Salmonella's Desiccation Survival and Thermal Tolerance: Genetic, Physiological, and Metabolic Factors

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Abstract

Salmonella can survive for long periods under extreme desiccation and low water activity conditions (a_w < 0.6) while becoming tolerant to heat. This stress tolerance poses a risk for food safety, but relatively little is known about the molecular and cellular processes involved in this adaptation mechanism and its potential for cross-protection.

This dissertation consists of three distinct studies focused on elucidating this mechanism. The objective of the first study was to identify the genes involved in *Salmonella*'s resistance to desiccation. A global transcriptomic analysis comparing *S. enterica* serovar Typhimurium cells equilibrated to low a_w (a_w 0.11) and cells equilibrated to high a_w (a_w 1.0) determined that 719 genes (16% of the total number of genes in the genome) were differentially expressed between the two conditions. The genes that were up-regulated at a_w 0.11 (290) were mostly involved in metabolic pathways, DNA replication/repair, regulation of transcription and translation, and virulence. Based on the transcriptomic analysis, we created deletion mutants for two virulence genes, *sseD* and *sopD*, and tested their ability to survive desiccation and low a_w on glass beads. The two mutants exhibited significant cell viability reductions after desiccation compared to the wild-type and additional decrease after exposure to a_w 0.11 for 7 days. Under scanning electron microscopy, the mutants displayed a different cell morphology and extracellular matrix production when compared to the wild-type under the

same conditions. The findings of this study suggested that *sopD* and *sseD* are required for *Salmonella*'s survival during desiccation.

The objective of the second study was to determine the effect of food and inert matrices, nutrient availability, and growth conditions on desiccation survival and thermal tolerance of *S. enterica* serovar Typhimurium. *Salmonella* was grown in LBglc and M9 media, in the presence or absence of EDTA and dipyridyl. Cultures were inoculated on toasted oat cereal (TOC) or glass beads, dried, and equilibrated for a week at a_w 0.11 and 1.0, before being thermally treated at 75, 85, 90, and 95ºC. For all growth conditions and temperatures tested, cells exposed to a_w 0.11 had inactivation rates (δ-values) at least 10-fold longer than cells equilibrated at a_w 1.0. Our results showed that growth in the presence of EDTA or Dipyridyl did not have any effect on *Salmonella*'s thermal tolerance at either a_w on TOC. In control conditions, recovery after drying and thermal tolerance was higher on TOC than on glass beads, suggesting that the food matrix was protective for desiccation and thermal treatment. Growth in M9 resulted in lower survival to drying and exposure to low a_w on glass beads, compared to LBglc. On the contrary, thermal tolerance increased in cells grown in M9 compared to LBglc at both a_w. Cells grown in LBglc and M9 displayed differences in the production of extracellular matrix, in particular during equilibration to a_w 0.11 and after thermal treatment at both a_w. Additionally, when *Salmonella* was grown on glass beads in LBglc as biofilm, the thermal tolerance was greater than free cells dried on beads. Our observations suggest that the

presence of nutrients during growth and before exposure to desiccation and thermal treatment influenced *Salmonella*'s ability to survive desiccation and develop thermal tolerance.

The objective of the third study was to identify proteins involved in *Salmonella*'s resistance to desiccation and thermal treatment using iTRAQ. Proteins were extracted from *S. enterica* servorar Typhimurium cells dried, equilibrated at high a_w (1.0) and low a_w (0.11), and thermally treated at 75°C. Our analysis determined that 734 proteins were differentially expressed among samples, and of these 175 proteins were the most significant in determining differences in the proteomic profiles among treatments. Based on their proteomic expression profiles, the samples were clustered in two main groups by PCA analysis, "dry" samples and "wet" samples, while we did not observe significant differences between the thermally treated samples and the non-heated samples, at both a_w. Protein profiles indicated shifts in cell metabolism in both samples, as well as a strict regulation of DNA repair, replication, transcription, and translation. "Dry" samples had higher levels of 50S and 30S ribosomal proteins, indicating that ribosomal proteins might be important for extra-ribosomal regulation of cellular response even when the synthesis of proteins is slowed down. Stress response proteins were more frequently present in "wet" samples compared to "dry" samples, including SspA, GorA, and Dps, suggesting that "wet" cells were activating stress systems in response to rehydration. In conclusion, our study indicated that pre-adaptation to dry conditions was linked to increased thermal

tolerance, while reversion from a dry state into a wet state implied a significant change in protein expression that is linked with reduced thermal tolerance.

Table of Contents

List of Tables

List of Figures

1. Literature Review

1.1. *Salmonella*

1.1.1. General and physiological characteristics

Salmonella is a Gram negative facultative flagellated anaerobic rod, approx. 2-3 μm long (1, 2), belonging to the Enterobacteriaceae family. Although most serovars have peritrichous flagella, some serovars of *Salmonella* are non-flagellated like serovar Pullorum and Gallinarum (1, 3). *Salmonella* is a chemotrophic bacterium that has both respiratory and fermentative pathways, and can transform glucose and other carbohydrates into organic acids and gas. *Salmonella* can also use citrate as a primary carbon source, it does not hydrolyze urea, and it is oxidase negative and catalase positive (3). Salmonellae are able to decarboxylate lysine and ornithine, and produce hydrogen sulfide. These biochemical traits are usually combined for the differentiation of *Salmonella* from other microorganisms.

Salmonella cells are able to survive and grow under different environmental conditions. *Salmonella* grows optimally at temperatures from 35 to 37ºC, but some strains can grow at temperatures from 2 to 54ºC (4). The optimal growth pH ranges between 6.5 and 7.5, but *Salmonella* can still grow at pH values of 4.5 and 9.5 (5). *Salmonella*'s ability to grow is influenced by water activity (a_w), a physical property determined by the amount of water available for biological and chemical reactions. Like most Gram negative bacteria, *Salmonella*

requires a a_w value of at least 0.96 to grow (5) but it has been demonstrated that it can survive desiccation and low water activity conditions ($a_w < 0.6$) for several months on food matrices (6). The combination of these characteristics allows *Salmonella* to tolerate multiple stresses, making it an ubiquitous microorganism.

1.1.2. Nomenclature

In the past decades, following the shift from biochemical and serological characterization to DNA homology and, recently, DNA sequencing, *Salmonella* taxonomic classification has been reorganized multiple times with its species redefined first as subgroups and later as subspecies, (5). According to the current classification, the *Salmonella* genus consists of only two species, *S. bongori* and *S. enterica*, and more than 2,500 serovars (1). *S. enterica* is divided in 6 subspecies, noted with Roman numerals, letters, and names (Table 1.1), while *S. bongori* only contains one species (7, 8). The species and subspecies are classified according to the Kaufmann-White-Le Minor scheme (1, 7), which is based on three major determinants: somatic O lipopolysaccharides (LPS) , flagellar H antigen, and capsular K antigen, which in *Salmonella* are limited to the Vi antigen and only present in the serovars Typhi, Paratyphi, and Dublin (9).

Table 1.1. *Salmonella* species, subspecies, and serovars*.

***Salmonella* species**	**Subspecies (name and Roman numeral)**	**Nº of serovars**
Salmonella enterica	*enterica* (I)	1,504
	salamae (II)	502
	arizonae (IIIa)	95
	diarizonae (IIIb)	333
	houtenae (IV)	72
	indica (VI)	13
Salmonella bongori	(V)	22

* Adapted from (5)

Salmonella is unique in that serovars were originally named through correlation of a syndrome, host specificity, or geographic location in which they were isolated, rather than based on their antigenic formula (2). The strains classified under *Salmonella enterica* subspecies *enterica* are of taxonomic importance. This subspecies of *Salmonella* is extremely diverse and represents 99% of the total isolates from humans (1, 3) Moreover, of the cases of salmonellosis in the United States, 70% are caused by only 20 of the 1531 serovars comprising *S. enterica* subsp. *enterica* (3, 10). Not surprisingly, several of these 20 strains have been implicated in outbreaks where a low water activity food or ingredient served as a vector for the bacterium. These include *S.* Enteritidis, *S.* Typhimurium, *S.* Agona, and *S.* Montevideo (3, 11).

1.2. *Salmonella* in the environment

1.2.1. *Salmonella* natural reservoirs

Similar to other enteric bacteria, *Salmonella* is a natural inhabitant of the gastrointestinal tract of animals, including mammals, birds, and reptiles. Most of the serovars are adapted to a broad range of hosts, while some serovars only colonize mammals. The serovar Typhi infects only humans. While the distribution of reptiles, particularly turtles, geckos, and snakes, as house pets has been increasing over the last decade (12), birds and mammals are still considered the most significant reservoirs of human health concern for *Salmonella*, mainly due their consumption as food products.

Poultry is the second-most frequently consumed meat worldwide (13) and because of their high consumption as food products, chicken and turkey are considered the predominant vehicle for transmission of *Salmonella* spp. The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) estimates put the prevalence of *Salmonella* in chicken carcasses at a maximum of 16% in 2005 decreasing in the following years to 4.3% in 2012 (13, 14). Similar studies have been performed throughout the world and demonstrate that the overall prevalence of *Salmonella* in poultry fluctuates depending on location. A large study conducted from 2000 to 2002 on Uruguayan poultry estimated that the overall prevalence of *Salmonella* among poultry in Uruguay was 6.3% (15). A four-year study conducted in Vietnam found a similar prevalence to that observed in United States at 4.60% (16). While both

of these studies yielded relatively similar results to what was observed by the FSIS, an examination of 1152 chicken carcasses obtained from different markets in China found that the prevalence of *Salmonella* in these samples was 52.2% (17). In a comparative study done in Spain in 1993 and 2006, the prevalence of *Salmonella* found within chicken samples purchased from retail outlets decreased from 55% to 12.4% (18).

As mentioned above, reptiles are another reservoir for *Salmonella*, and they are becoming increasingly important for the spread of this bacterium in household environments, and exposing kids to the infection. According to the CDC, between January 2015 and April 2016, four outbreaks of salmonellosis, involving 133 people in 26 states, were linked with handling of turtles (19). Similarly, between January 2014 and June 2015, 22 people in 17 states were infected with *Salmonella* associated with geckos (20). Wild and farm mammals are also important reservoirs of *Salmonella* and can be affected by infections with *Salmonella*. A 6 year-long study conducted in a northeastern dairy farm from 2004 to 2010 found that *Salmonella* infections were very often asymptomatic in cows and that, although shedding ranged from 8% to 97% of the herd, more than 50% of the herd was positive for *Salmonella* shedding for the duration of the study (21). Similar variation was also found in pig populations from two different swine production sites in North Carolina where fecal prevalence ranged from 0% to 48% (22).

1.2.2. *Salmonella* spread in natural environments

As a zoonotic microorganism, *Salmonella* can cycle between animals, plants, humans, and the environment (Fig 1). For this reason, knowledge of its environmental distribution and different factors influencing its environmental fitness is extremely important for the scientific community. As previously mentioned, wild and domesticated animals are natural reservoirs for *Salmonella*. In farms, *Salmonella* can be transmitted between animals by contaminated feed and by infected and asymptomatic individuals. In this environment, infected cattle can transmit the pathogen to the rest of the herd through feces (23), feed (24), and water (25). Many studies conducted on cattle operations, pig farms, and slaughterhouses detected *Salmonella* in animal feces as well as on the farm property and the surrounding environment, suggesting a widespread persistence (26-28).

One of the main sources of *Salmonella*'s environmental contamination is the use of animal waste for farming purposes. Studies performed on pig and cattle manure reported that the occurrence of *Salmonella* can vary from 1% to more than 31% (29). Additionally, *Salmonella* is able to survive for very long periods outside a host, and can tolerate different environments. A 2013 study of *Salmonella* in soil found that *S*. Newport was 100% recoverable from the tomato rhizosphere 23 days after inoculation, although *S*. Typhimurium was not recovered (30), suggesting that environment survival and adaptation was different among serovars. Many physical parameters can influence *Salmonella*'s

persistence and recovery in soil, the most important being moisture, temperature, and time from contamination (31). Mode of contamination and presence of different predatory protozoa can also affect the presence and detection of this pathogen in the soil (32).

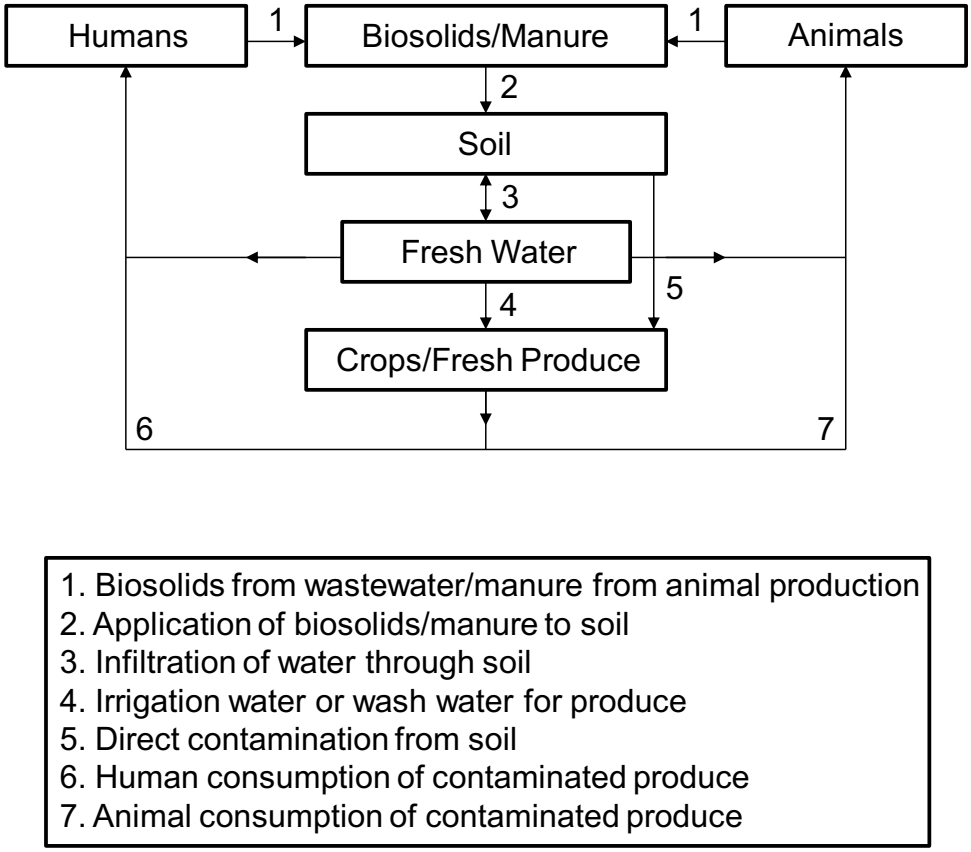


Fig 1.1. Schematic representation of Salmonella sources and routes of environmental spreading. Adapted from Jocobsen and Bech 2012 (29)

Once *Salmonella* is in the ground, it can cycle back to the animal host through water infiltration or contact with crops and feed. Plants can also host and harbor *Salmonella* and be responsible for its spreading to humans and animals.

Salmonella's first interaction with plants happens at the plant tissue surfaces. From there, *Salmonella* has to cope with different stresses during the process of internalization, due to the diverse environments that exist inside a plant. *Salmonella*'s ability to grow on and in plants changes depended on external environmental conditions, such as temperature and humidity (33-35). O'Brian and Lindow reported that the bacterial population increased in warm and humid conditions, while it remained static or decreased under dry conditions (33).

1.2.3. *Salmonella* in food processing environments

Because S*almonella* is a zoonotic bacterium, it is a frequent contaminant of raw animal foods, but it can also be found in many crops due to its presence in soil and water, where it can remain viable for years (5). Contaminated raw materials often serve as vehicles for *Salmonella* to easily access food processing and production plants. Several studies have indicated that *Salmonella* contaminated equipment surfaces and processing machines. A study published in 2013 reported that the same strain of *S*. Agona was responsible for two multistate outbreaks in 1998 and 2008, indicating that the strain remained present in the processing facility for 10 years (36). Among the main reasons for *Salmonella* outbreaks are also cross-contamination (37), and recontamination of food after thermal inactivation processes (38). Instances of cross-contamination are rather frequent because of the capability of this pathogen to survive for long periods on inert food contact surfaces, such as plastic, stainless steel and glass surfaces (39-44).

Many studies have shown that the *Salmonella*'s persistence in processing facilities is due to its ability to withstand harsh environments and treatment. Moreover, it has been demonstrated that pre-exposure to certain sublethal stresses, including acid, temperature, and desiccation stress, often results in cross-protection against successive treatments (3). For example, acid-adapted cells of *S*. Typhimurium exposed to pH 4.8 showed an enhanced survival in mildly acidic orange juice (pH 3.6) at 25 and 37ºC (45). A different study demonstrated that lactic-acid adapted *S*. Enteritidis at pH 5.3 and 6.3 could better withstand exposure to extreme acid conditions (pH 2.0) and had higher heat resistance (46).

The formation of biofilms poses an additional problem when *Salmonella* colonizes food contact surfaces (47-51). Biofilms are well known to be a persistent source of contamination and difficult to eliminate during standard cleaning and sanitation procedures. Surface properties (i.e., type of material, shape and configuration of the surface) influence frequency of bacterial attachment, efficacy of biofilm formation, and resistance to sanitizing agents (52, 53). A study from Korber *et al*. showed that treatment with trisodium phosphate, a common ingredient of sanitizers, removed more cells from a 48 h old biofilm then from a 72 h biofilm, suggesting that biofilm's age also impacts the efficacy of sanitization (52). Different strains of *Salmonella* also have different biofilm formation properties. *Salmonella* Typhimurium ATCC 23564 and 19585 produced more biofilm than ATCC 14028 on polystyrene microtirer plates (54).

1.3. Foodborne *Salmonella* – Epidemiology

1.3.1. Disease characteristics

Salmonella is a foodborne pathogen capable of causing enteric and systemic infections. Salmonellosis is a general term that refers to three kinds of ailments: enteric fever, enterocolitis, and systemic infections. Enteric fever is caused exclusively in humans by the serovars Typhi, Paratyphi, and Sendai (55), while gastroenteritis is caused by non-typhoidal strains. Typhoid fever is due to ingestion of contaminated water or foods, as well as close proximity to an infected individual (55). In humans, the incubation time is about 2 weeks, after which fever and illness manifest. It is usually associated with abdominal pain, and occasionally with nausea, headache, myalgias and constipation. In case of immunocompromised patients, diarrhea can also manifest as a symptom. Although the disease often resolves itself after weeks, the infection can be silent in patients, months or years after the symptoms disappear, and in some cases it can relapse (56).

Ingestion of non-typhoidal serovars, typically greater than 50,000 bacterial cells (55), causes enterocolitis. This is usually characterized by a variety of symptoms, such as abdominal pain, diarrhea, with or without blood, nausea, and vomiting. Symptoms typically develop from 6 to 72 hours after exposure and are usually self-limiting, lasting between 5 and 7 days (57). In certain cases, such as immune-compromised patients and elders, bloodstream infection can occur and lead to systemic infections, septicemia and the death of the patient. *Salmonella*

has also been linked to other serious conditions, such as meningitis and osteomyelitis (58), reactive arthritis and ankylosing spondylitis (59, 60).

1.3.2. Prevalence and incidence

Epidemiology and host range of non-typhoidal *Salmonella* varies among serovars. While serovar Typhimurium is considered a host generalist, with a broad host range and low chance of causing systemic infection, other serovars, like Dublin and Heidelberg, are more likely to spread into the bloodstream and cause invasive infections (61). On the other hand, for example, Typhimurium has a higher case fatality ratio than Newport (62, 63). The factors that determine these differences among *Salmonella* serovars are complex and not completely understood yet. One factor that has been linked with differentiation among strains is gene decay, in particular of metabolic and virulence genes, which seems to influence infection mechanisms and host specificity (64, 65).

In developed and industrialized countries, animal products and contaminated produce are the main source of non-typhoidal *Salmonella* (66), while in developing countries waterborne transmission and person-to-person transmission play a more important role (67). Indeed, a study performed in Kenya on pediatric cases from 2002 to 2004 reported that 69% of human contacts of the index patients carried *Salmonella* in their stools, and that 66% of the isolated strains were similar to those of the index patients (68). Further analysis of the household environment and livestock found only unrelated strains, suggesting that the transmission route was most probably person-to-person (69). In

developing countries, other factors, such as coinfections with HIV and malnutrition, contribute to the prevalence of the disease (69). For example, non-typhoidal *Salmonella* in Africa has been shown to be seasonal, with peaks of infection during the rainy season (70), which also coincides with a higher incidence of malaria and malnutrition (69).

On a global scale, two reports have estimated that there is an average of 78.4 million (71) to 93.8 million (72) cases of non-typhoidal *Salmonella* per year. Majowicz *et al* estimates that 86% (80.3 million) of these illnesses are foodborne in origin. Moreover, this bacterium is also responsible for an estimated 59,153 (71) to 155,000 (72) deaths globally every year. In 2010, according to Ao *et al*, non-typhoidal *Salmonella* caused about 3.4 million invasive infections and 681,000 deaths, 57% of which occurred in Africa (73). According to the Center for Disease Control and Prevention (CDC), non-typhoidal *Salmonella* is responsible for approximately 1.2 million illnesses and more than 450 deaths each year in the USA (74). A study published in 2011 by Scallan *et al* identified non-typhoidal *Salmonella* serovars as the leading cause of bacterial foodborne illness, being responsible for 11% of the total foodborne illnesses every year and being the leading cause of hospitalizations (19,336, 35% of the total foodborne related hospitalizations) and deaths (378 cases, 28% of the total foodborne related deaths) (75).

In 2015, the CDC's FoodNet identified 20,107 confirmed cases in 10 US cities, with *Salmonella* being responsible for 38% (7,728) of those and was,

therefore, the foodborne prokaryotic pathogen with the highest prevalence (76). Of the 7,728 cases of salmonellosis, 2,074 (27%) lead to hospitalization, and 32 (0.4%) resulted in death. In 2013, the Economic Research Service of the USDA estimated between 644,786 and 1,679,667 cases of salmonellosis in the US, with a mean of 1,027,561 cases, for a total economic burden of more than 3.6 billion dollars (77). In a report published in 2015, the monetary loss per case of non-typhoidal salmonellosis was estimated to average $5,337, with a full monetary loss due to all non-typhoidal *Salmonella sp*. of 5.5 billion, which exceeds all the other foodborne illnesses, due to its higher annual occurrence (78).

1.3.3. Outbreaks

Salmonella infections have historically been linked with the consumption of food of animal origin, such as beef, poultry, pork, and eggs (1, 79-83). Notable outbreaks implicating foods of animal origin include the consumption of stuffed ham contaminated with *Salmonella* Heidelberg which resulted in 746 illnesses (84) and the consumption of stuffed chicken contaminated with *Salmonella* Hadar, which led to 2,138 illnesses (85). These outbreaks were often related to the consumption of food that was not properly heat-treated and, for this reason, general guidelines exist to heat animal products to inactivate pathogens. *Salmonella* contamination is also associated with dairy products and several outbreaks have been associated with the consumption of contaminated milk, cheese, and ice cream (3). One of the largest outbreaks of *Salmonella* documented in the US occurred in 1985 and was related to the consumption of

contaminated pasteurized milk which sickened over 16,000 individuals (86). The actual cause of contamination was never determined, but it was speculated that pasteurized milk was contaminated with raw milk during cooling (86). While major outbreaks implicating animal products have been a major concern of the food industry, a growing number of outbreaks have been linked to foods not traditionally considered a risk for contamination with *Salmonella* including both fresh produce and dry foods.

In the last 20 years, *Salmonella* outbreaks have been associated with the consumption of a wide range of fresh produce including cantaloupes, tomatoes, sprouts and peppers (57). The most common serovars associated with human infections are *S*. Enteritidis, *S*. Typhimurium, *S*. Newport, *S*. Javiana, *S*. Montevideo and *S*. Heidelberg (10). Cucumbers have also been implicated in major *Salmonella* outbreaks including a *Salmonella* Poona outbreak in 2015 and 2016, where 907 cases were documented, including 6 deaths. The cucumbers were imported from Mexico and the epidemiological investigation did not identify whether contamination occurred during shipping or was due to poor farming/harvesting practices at the farm level (87).

Sprouts are also a common vector for disease spread and have been associated with several *Salmonella* outbreaks including two occurring in 2016 due to the consumption of contaminated alfalfa sprouts (88, 89). Likely production and processing of the sprouts is implicated in the high propensity for contamination of this crop. It has been reported that alfalfa can not only support

high levels of bacteria (as high as 10^9 CFU/g) (90), but can also sustain large *Salmonella* counts (as high as 10^6 CFU/g) (91). Seeds are germinated in warm, moist conditions and, as a result, if a pathogen is present on the seeds before germinating, the conditions are ideal for the pathogen grow and proliferate.

1.3.3.1. Low water activity (a_w) foods outbreaks

Specific to the focus of this project is the nature of *Salmonella*'s survival in a dry environment. Until recently, dry foods were considered largely safe because of their supposed lack of sufficient water to sustain growth, leading to a decrease in the viability of bacteria. Foods with a measured water activity (a_w) lower than 0.60 are considered low a_w. As a result, foods such as dark chocolate, potato chips, spices, nuts, crushed red pepper, peanut butter, crackers, flour, and cookies, all of which have been implicated in *Salmonella* outbreaks and recalls over the past 5 years, can be included in this category (92). While the number of recalls and outbreaks related to these products has been increasing in recent years, it is likely that many cases previously went undetected due to the difficulty of tracing products used sporadically across several meals (92). The increased rate of *Salmonella* contamination events occurring in low a_w foods is likely due to improvements in epidemiological investigations and increased sensitivity of testing methods used to detect the pathogen.

The first documented case of a *Salmonella* outbreak linked to a low water activity food occurred in 1985 where 62 individuals (46 infants and 16 who were in contact with infants that consumed the product) were sickened by

contaminated infant formula (93). Since then, 6 additional *Salmonella* outbreaks have been linked to infant formula (94-97). Other low water activity foods have also been implicated in *Salmonella* outbreaks including the consumption of cake-batter ice cream (98) and wheat flour (11). The raw flour outbreak of 2008 and 2009 was particularly noteworthy as it was the first identifying wheat flour as a potential vector for *Salmonella*. The flour outbreak also illustrates another common theme observed regarding the nature of these outbreaks. Contaminated flour was used as a baking ingredient and the consumption of a raw baking mixture caused the infection of several individuals (11).

Some of the largest and therefore most impactful *Salmonella* outbreaks traced back to contaminated low water activity have been those that occurred from the consumption of nuts and nut-containing products. Among several outbreaks that have been documented, two major outbreaks occurred, the first from 2006 to 2007 and the second from 2008 to 2009, which sickened over 1400 individuals (99, 100). One of these two outbreaks, a peanut butter outbreak involving the Peanut Corporation of America (PCA), affected 714 individuals in 46 states (100). Those confirmed cases were just the individuals that sought medical attention, but estimates put the number of actual cases much higher since many people did not seek hospitalization or their illness was not determined or recorded. Indeed, one estimate of the actual case numbers for this outbreak was 16 times greater than the number of documented cases (101). This would equate to over 11,000 cases. The occurrence of these outbreaks clearly

proved that dry foods were potential sources of contamination and made the scientific community aware of this potential public health threat.

1.4. *Salmonella*'s pathogenicity

1.4.1. Infection mechanism

Salmonella infection starts with the ingestion of contaminated food or water, followed by its survival through the gastric environment to reach the small intestine. Once there, the pathogen enters the single-cell epithelial layer of the intestine to gain access to the host tissue and establish an infection (102). Most of the internalization occurs in the distal ileum (103) mainly at two sites: villi and Peyer's patches (PPs). Although the translocation to the *lamina propria* through the epithelium covering the villi is possible, the PPs are the preferred sites (104). Indeed, the follicle-associated epithelium of the PPs, and specifically the phagocytic microfold cells (M cells) have a more accessible cellular architecture, since they consist of a single layer of epithelial cells and the lack of microvilli and the thick layer of mucus (104, 105). Another possible invasion pathway is through direct uptake from dendritic cells (DCs), which can be present in the follicle-associated epithelium (106) or can be recruited during the infection (107).

Salmonella can be taken up from the DCs through the dendrites that penetrate between adjacent cells (108-110). Once *Salmonella* has exited the intestinal lumen through M cells uptake (in the PPs) or direct dendritic uptake, it reaches the mesenteric lymph nodes (MLN) (102), where it has the opportunity to

spread to different organs, such as the spleen and liver. Phagocyte-associated *Salmonella* can be found in the blood stream within a few minutes after the infection (111, 112). This suggests that *Salmonella* can reach the blood stream directly by leaving the MLN as a single cell, but the mechanism is yet to be elucidated.

After *Salmonella* invasion, the phagocytes present in the intestinal lumen, in particular macrophages, are the first component of the innate immune response to encounter the bacterium (113-115). Pathogen-associated molecular patterns (PAMPs) are recognized by Toll-like receptors (TLRs) and the pattern-recognition receptors (PRRs) on the vacuole membrane (116). It has been shown (117) that recognition of *Salmonella*'s PAMPs by TLRs is essential to induce acidification of the vacuole, which triggers the secretion of effectors by *Salmonella,* and eventually replication of the pathogen and formation of the *Salmonella* containing vacuole (SCV), which is essential for intracellular growth.

Salmonella has developed a series of cellular responses to thrive in the host. These mechanisms serve mainly two purposes. The first one, and more immediate, is to ensure the survival of the pathogen in the host. This response is regulated to confer the bacterium tolerance to the stresses encountered during the invasion process, such as acid stress in the stomach and oxidative stress in the macrophage. The second, instead, is to ensure the initiation, as well as the continuation, of the infection process, thanks to the release of effectors that manipulate the host cellular functions, biochemistry, and physiology (118). These

mechanisms involve numerous players, which are defined as virulence factors.

1.4.2. Virulence factors

Most of the genes involved in the initial infection are encoded by the *Salmonella* pathogenicity islands (SPIs), several discrete regions of the genome characterized by a lower G+C content, a sign of horizontal genetic transfer (119), and which contain most of the virulence genes (120). Many SPIs have been identified in the last years, but their role during the infection process is not well understood yet. The main SPIs that are involved in the invasion mechanisms are SPI1 and SPI2, both encoding for type 3 secretion systems (T3SS) essential for the invasion process (118).

The T3SSs are injection machineries that allow the bacteria to insert effectors in the host cell, start the internalization process and facilitate the infection (121, 122). SPI1, a 40-kb DNA region, encodes for a T3SS that is strictly regulated in response to different environmental stimuli (118) and that is fundamental for the intestine colonization, while it is not expressed after the internalization (119). The T3SS encoded by SPI1 injects effectors in the cytosol of the host epithelial cells, causing rearrangements of the cytoskeleton, which eventually trigger the engulfment and phagocytosis of the pathogen (123-125). SPI1 is responsible for secretion of flagellin into the cytosol of the host cell, thus activating the NLRC4 inflammasome response (126) and inducing pyroptosis, a kind of cellular death. Although cell death can work as a host defensive mechanism to control pathogen replication, *Salmonella*'s induced pyroptosis in

intestinal cells, instead, represent a way to facilitate systemic infection, since bacterial cells are released from the dead cells of the monolayer (127).

The master gene of the main SPI1 regulators (e.g. HilA, HilC, HilD, InvF, and SprB) is *hilA* (128-131). This regulator acts as transcription activator, binding directly to *prgH* and *invF* promoters and activating the expression of the *prg/org* and *inv/spa* genes (132, 133). Deletion of *hilA* causes a phenotype very similar to a SPI1-deleted strain (134), thus suggesting that HilA controls the activation of all components necessary for a functional SPI1-T3SS (134). In turn, *hilA* transcription is positively regulated by HilC, HilD, and RtsA in response to different environmental conditions that are usually found after the invasion of the host, such as low O_2 concentration, mildly acidic pH, and high osmolarity (104, 135-137).

SPI2 is present in *S. enterica* but absent in *S. bongori*, and is probably the main step in the evolution of *Salmonella enterica*'s ability to cause systemic illness. (138). It was reported that *Salmonella* mutants lacking SPI2 are not able to proliferate inside the host organs (139), thus suggesting that SPI2 genes are essential for the establishment of systemic diseases (140-142). SPI2 genes are involved in *Salmonella* response to reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by the macrophage (143, 144). ROS are produced after neutrophil recruitment and the activation of NADPH oxidase complexes, which transfer electrons from NADPH to O_2, thus generating oxygen radicals (116). One of the most common RNS is nitric oxide, which results from

the conversion of L-arginine by an inducible nitric oxidase synthase (iNOS) present in the intestinal epithelial cells, macrophages, and neutrophils (145).

While ROS and RNS work as a defensive response from the host, due to the cellular stress (mostly oxidative) that they induce in the pathogen, it is important to remember that those species have stronger toxic effects on other microorganisms which are part of the natural microbiota, such as Clostridiales and Bacteroidetes, rather than on *Salmonella*. Indeed, the production of these compounds partially helps *Salmonella*, reducing competition by other bacteria, and creating highly oxidative environments in which *Salmonella* can use alternative anaerobic electron acceptors (146-150). For example, it has been shown that ROS generated during the infection react with sulphur compounds present in the lumen and form tetrathionate, which *Salmonella* can use as an electron acceptor, differently than competing microbiota (149).

Moreover, one of the main host responses to infection is the production of lipocalin-2 and calprotectin by epithelial cells and neutrophils (148, 151, 152). Lipocalin-2 can sequester the siderophore enterochelin, a small chelator with high iron affinity released by *Enterobaceriaceae* into the environment to scavenge iron (153). *Salmonella* produces an additional siderophore, salmochelin, that cannot be bound by the lipocalin-2 and therefore can escape iron starvation (154). Similarly, *Salmonella* possesses a high affinity zinc transporter, ZnuABC, which allows the bacterium to overcome zinc sequestration by calprotectin (155), a heterodimeric protein abundant in neutrophils, and

released in high quantities during inflammation (156).

SPI1 and SPI2 are functionally connected, and belong to a complex regulatory network. For example, the histidine kinase PhoQ, part of the PhoP/Q two-component system, responds to changes in extracellular cation concentrations (157) and is fundamental for SPI1 regulation. When the Mg^{2+} levels are low, such as inside macrophages, PhoQ phosphorylates PhoP, which then activates the expression of genes of the SPI2, required for the intra-macrophage survival (158). PhoP overexpression has been shown to cause a decrease in *hilA* expression confirming that regulation of invasion genes by PhoPQ is mediated by regulation of the master regulation hilA (135).

Iron concentration is also an important factor for *Salmonella*'s virulence. During infection, *Salmonella* faces very different iron concentrations. Generally, the concentration of free Fe^{2+} in host tissue is very low due to the sequestration mechanisms activated by the host cells as defense (159, 160). However, in the lumen of the small intestine, where most of the dietary iron is absorbed, there is an abundance of free Fe^{2+} (161). It has been reported that many *Salmonella* genes are silenced if the bacterium remains in the lumen, suggesting that the concentration of free Fe^{2+} plays an important role as a signal for the bacterium to sense the physico-chemical characteristics of the environment conducive to its successful colonization (162, 163). The ferric uptake regulator (Fur) is one of the main systems that the cell possesses to regulate intracellular iron concentration. Fur is a 17-kDa homodimeric protein, which can bind DNA when associated with

its cofactor ferrous iron (Fe^{2+}) and to repress transcription of many iron-uptake genes, binding to their operator sites (164, 165). The expression of *fur* can be induced by SoxRS system and OxyR, in response to oxidative stress (166).

For a long time, Fur has been considered a transcriptional repressor. However, not long ago scientists have recognized its role as a positive regulator of genes involved in various regulatory mechanisms. Fur-positive regulation occurs through the repression of the synthesis of the small RNA *ryhB*, a post-transcriptional regulator able to induce RNase E-dependent degradation of the target mRNAs thanks to RNA-RNA interaction between the mRNA target and the antisense small RNA(167-173). Moreover, it was recently discovered that *ryhB* can also act independently from the RNase, as a translational regulator by hybridizing and hindering the Shine-Dalgarno sequence, thus preventing the binding of the ribosome to the mRNA (174).

Iron concentration has been associated with the expression of the *Salmonella* Pathogenicity Island 1 (SPI-1) type 3 secretion system (T3SS) (162), and Fur has been shown to activate expression of SPI1 through the increase of HilD (162, 175). Moreover, it was recently observed that Fur also regulates activation of *hilA* by modulating the level of the histone-like protein H-NS (176). H-NS is a widely distributed and well-conserved protein in bacteria, it is encoded by the gene *hns* (177, 178) and plays an important role in iron uptake. H-NS, indeed, controls gene expression by binding to A-T rich DNA sequences and repressing transcription (179-181). In the presence of iron, Fur binds to the

promoter region of *hns* thus repressing it. Since H-NS inhibits the expression of *hilA*, the negative regulation of *hns* by Fur results in the activation of *hilA*, which eventually leads to the activation of the SPI1genes (176).

1.5. Water activity

1.5.1. Principles

All foods contain water in different amounts. Many parameters describe the presence of water in foods, including equilibrium relative humidity (ERH), moisture content, solute concentration, and osmotic pressure (182). Water content and solute concentration are not fully adequate parameters since they do not adequately describe the properties of the water available in a certain food product. On the contrary, osmotic pressure and ERH are more accurate descriptors of water available for reactions, but the first is based on the assumptions of the presence of a permeable membrane, which is not always true for food products. ERH refers to the equilibrium strictly between atmosphere and food, and not to the food itself (182).

The water content in foods has been described using the concept of water activity (a_w). a_w best defines water content since it includes cases when food is not in equilibrium with the atmosphere and better describes water in relation to its availability for chemical and biological reactions (182, 183). To fully understand what a_w is, it is necessary to start from the concept of ideal solutions, entropy, and the Raoult's law. When a solute is added to a solvent, like water, the entropy

of the system decreases, since the molecules of solvent are less free to escape from the solution into the vapor phase. As a result, the vapor pressure of the solvent – that is the pressure at which the vapor phase of the solvent is in equilibrium with the solvent – decreases as well. Raoults' law describes this relationship and states that the lowering of vapor pressure of a solvent when adding a solute is equal to the mole fraction of the solute, and is expressed as:

$$\frac{p_0 - p}{p_0} = \frac{n_1}{n_1 + n_2}$$

(1)

where p_0 and p are the vapor pressure of the solvent and the solution, respectively, and n_1 and n_2 are the number of moles of the solute and solvent, respectively. Raoults' law can also be expressed as:

$$\frac{p}{p_0} = \frac{n_2}{n_1 + n_2}$$

(2)

The ratio of the vapor pressure of a solution (p) and the vapor pressure of the pure solvent (p_0) is described as water activity (a_w), so that:

$$a_w = \frac{p}{p_0}$$

(3)

Being directly connected with vapor pressure, a_w is linked to the moles of solute in the state that the solute behaves in the solution. Thus, a_w is a much better descriptor than moisture content, especially for complex systems like foods. For example, while the moisture content of egg yolk with 10% sucrose or 10% NaCl is the same, the a_w of egg yolk with 10% salt is lower than the a_w of egg yolk with 10% sugar, due to the way the salt molecules dissociate in the water of the egg yolk (184). In equilibrium, ERH is equal to a_w multiplied by 100 and pure water has an a_w of 1.00 and an ERH of 100%. It is important to remember, though, in foods, solutes do not behave ideally, and therefore the reduction in vapor pressure can be greater than what is predicted by Raoults' law, and the same is true for a_w (182).

1.5.2. Water activity and foods

As above mentioned, a_w is a critical factor in determining the rate of enzymatic and biological reactions. Therefore, in food products a_w is deeply connected with the concepts of shelf life, food spoilage, and food safety. The rate of some of the most critical enzymatic reactions in foods, such as Maillard reactions and lipid oxidation, are controlled by a_w. Moreover, a_w is a vital indicator for microbial growth. Most bacteria can grow only when a_w is higher than 0.88-0.91, with the exception of halophilic bacteria, which can grow when a_w is as lower as 0.75 (5). Most yeasts have a minimum a_w requirement of 0.88, but is even lower for osmophilic yeasts (0.60), while most molds require an a_w of 0.80, but xerotolerant molds can grow at an a_w as low as 0.71 and xerophilic molds as

0.62 (185).

One of the main strategies to control bacterial growth in foods and on surfaces is through the reduction of the available water (186) to create a low moisture environment (< 13%) (187), because low a_w foods (< 0.60) have been considered safe from pathogen contamination. However, in recent years, many low a_w foods (i.e., black and red pepper, peanut butter, rice, cereals, chocolate, dry milk) have been associated with salmonellosis outbreaks (188-190), thus suggesting that low a_w conditions are not sufficient for *Salmonella* control. Many studies have shown *Salmonella*'s ability to survive on different dry food matrices for long periods, ranging from weeks to months (6, 191-193). For this reason, dry conditions alone can no longer be considered a method to guarantee safety and avoid contamination from *Salmonella*.

1.6. Low a_w and thermal tolerance in *Salmonella*

1.6.1. Survival at low a_w

Outbreaks of *Salmonella* linked to dry food matrices have illustrated that not only is *Salmonella* capable of survival in low water activity conditions, but can also survive in this environmental condition for extended periods of time. The investigation of a *Salmonella* Napoli outbreak related to the consumption of chocolate bars ($a_w \approx 0.4$) revealed that *Salmonella* was still detectable in chocolate bars for up to 12 months after the date of manufacture (194). It is important to note that the matrix, whether an inert surface or a dry food such as

flour, plays an important role in the ability of *Salmonella* to survive under desiccation stress. In the case of a complex matrix, such as food, the extrinsic environmental conditions to which *Salmonella* is exposed are many and diverse, such as variations in pH, in salt and in sugar concentrations. Fat content is an important component when considering the food matrix characteristics. Low a_w-high fat content foods, such as peanut butter, form a colloidal suspension in which water forms reverse micelles (water-in-oil micelles), droplets of water dispersed in the organic component of the matrix. When inoculated in these kind of matrices, bacterial cells tend to aggregate close to the water-oil interface, and therefore their survival may be influenced by the size of the water micelles (195).

Salmonella is able to survive for extended periods when desiccated even when nutrient sources are limited. A study of *Salmonella* survival kinetics on stainless steel discs revealed that the pathogen is able to survive for at least 30 days when held at 25°C following inoculation (196). It is also important to note that after an initial reduction observed during the first 72 hours of exposure, the overall numbers did not decrease any further (196). A study by Hiramatsu *et al* also demonstrated that, while sodium chloride had a negative effect on the desiccation tolerance of *Salmonella* on paper disks likely due to the disruption of osmotic balance when salts are concentrated during drying, an increase in the level of sucrose in the disks translated to enhanced survival (up to 79-fold) (197).

Temperature is also an important factor that influences the ability of the organism to survive desiccation, on both abiotic and food matrices. When dried

on paper disks at lower temperatures, the organism is incredibly difficult to inactivate as noted by Hiramatsu *et al*. who demonstrated that all the 5 tested strains of *Salmonella* were able to survive for at least 70 days at 4°C, but only 35 and 15 days when stored at 25 and 35°C, respectively (197). In peanut butter, a five-strain cocktail of *Salmonella* was detectable for the duration of the study (24 weeks) at 5°C when the inoculum level was 1.5 Log(CFU/g) (191), but the pathogen was undetectable after the same storage time when held under ambient temperature (21°C). The importance of storage temperature on the survival of *Salmonella* when desiccated was also demonstrated on almonds (198) and pecans (199).

The ability of *Salmonella* to survive under desiccation also extends to low water activity food products such flour and milk powders. Not only can *Salmonella* survive for long periods of time in these matrices, but the water activity of the matrix will also influence the ability of the organism to survive desiccation. A general survival study of *Salmonella* equilibrated to three a_w (0.33, 0.53, and 0.81) on skim milk powder demonstrated that *Salmonella* survived better at 0.33 and 0.53 a_w compared to 0.81 when stored for 2 months at 37°C (200). Another study of whey protein powder inoculated with *Salmonella* revealed a statistical difference between the survivability of *Salmonella* when exposed to 0.18 and 0.54 a_w (201). However, results were not consistent among strains, and some strains were more capable of survival at one water activity versus the other. This is an interesting notion, as it has also been observed that strain type

plays an important role in desiccation survival. In the same study, *Salmonella* Tennessee recovery was statistically greater than both *S*. Typhimurium and *S*. Montevideo (201).

1.6.2. Thermal inactivation

Thermal treatment of food products with the purpose of cooking, improving food safety and promoting preservation has been used for a very long time. The first examples of food thermal processes for packed items in sealed containers are dated back to the early 19th century. At that time, Napoleon's troops had very poor diets eating mostly badly salt-cured meat that lacked nutrients and was often the cause for scurvy. To solve this problem, Napoleon offered a monetary reward to the person who could develop a safe food-preservation method. One of the competitors, the French chemist Nicolas Appert, observed that food heated inside of a sealed container remained stable and preserved as long as the seal was not broken. Appert patented his thermal treatment process in 1810 (202). After that, in the late 19th century, a low-temperature heating process referred to later as pasteurization was developed by Louis Pasteur, with the purpose of controlling spoilage of alcoholic beverages. The invention by Appert set the foundations for the development of modern canning processes, which are still used today and are a fundamental tool in control of food preservation.

1.6.2.1. Inactivation kinetics

Microbiologists have developed different mathematical models for describing microbial inactivation. Mostly, these models are descriptive

mathematical simulations that describe the behavior of a system so that the performance of future systems can be predicted. These models differ from the mechanistic models, which instead aim to describe a complex system starting with the understanding of single factors in the system (203). While mechanistic models can account for specific environmental factors, the descriptive models of inactivation kinetics are generally preferred (202).

The first mathematical formulation of thermal inactivation was described by Bigelow et al. in 1920 (204), and it was applied to the study of bacterial spores. In 1922, Esty and Meyer (205) observed that the decrease in population in *C. botulinum* during thermal processing was exponential in time, due to the dependency of the inactivation on the initial number of microorganisms. Although the mechanisms of inactivation of spore and vegetative cells are biologically different, today studies of inactivation of microorganisms still rely on linear models and use the D-value, or decimal reduction time, as the most common inactivation rate parameter defined as the time required to reduce one logarithm the initial population. The equation that describes this relationship is:

$$\log\left(\frac{N_t}{N_0}\right) = -\frac{t}{D} \quad (4)$$

where N_0 is the initial population, N_t is the population at time t, and D is the decimal reduction time, so that –(1/D) is the slope of the regression line (202).

This equation can also be written as a first order kinetics equation, introducing the rate constant k:

$$\log\left(\frac{N_t}{N_0}\right) = -kt$$

(5)

where $k = \frac{\ln(10)}{D}$ (202).

Another important parameter in thermal inactivation is the *z*-value, defined as the increase in temperature necessary to decrease the *D*-value of a factor of ten. The *z*-value can be described graphically by the following equation (206):

$$z = \frac{T_1 - T_2}{LogD_1 - LogD_2}$$

(6)

where *D* can be expressed in second or minutes, and *z* is expressed in Celsius.

1.6.2.2. Non-linear models

The linear models described above were initially formulated with the intention of assuring inactivation of *C. botulinum*. When applying thermal inactivation to other microorganisms, it is important to consider that other species have different characteristics and, therefore, their inactivation rates might be different. The first order kinetics equations are based on the assumption that all the cells in a population have at any moment the same probability of survival, or

dying, which biologically means that a single cell is inactivated by a single effector.

This assumption is not always correct, in particular because of genetic and phenotypical heterogeneity, such as cell dimension, among the cells of a bacterial community can cause nonlinear behavior (207, 208). The most common deviations from a line model are referred to as “shoulders” or “tails” at the beginning or the end, respectively, of the inactivation process, indicating a higher survival of cells in specific moment of the process. In some case sigmoid curves are observed, in which both “shoulder” and “tail” are present before and after a linear inactivation period (202).

One very common non-linear model is based on the Weibull equation (209-214), which can describe both upward and downward inactivation curves. Many derivatives of the Weibull equation have been formulated, and Weibull-like equations have been used for modelling inactivation of both spores and vegetative cells. An example is the equation proposed by Mafart *et al*. (212):

$$\log\left(\frac{N_t}{N_0}\right) = -\left(\frac{t}{\delta}\right)^p \quad (7)$$

where N_t is the number of cells at time t, N_0 is the initial number of cells, t is the time of exposure to heat, δ is the scale parameter, and p is the shape parameter. The shape parameter p dictates the shape of the inactivation curve (Fig 1.2), and it is greater than 1 when the curve is concave downward and less than 1 when

the curve is concave upward. When the curve is linear, the shape parameter equals 1. In this case, the probability density function of the three-parameter Weibull equation reduces to that of the two-parameter exponential distribution, and therefore, the scale parameter δ, which resembles the inactivation constant k, becomes identical to the *D*-value. This is true also in cases when $p \neq 1$, but only for the first decimal reduction.

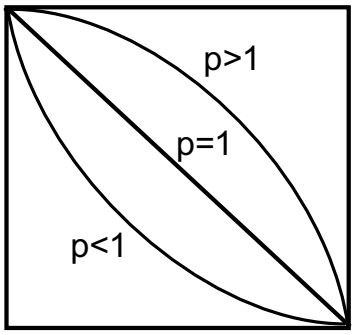


Fig 1.2. Schematic representation of the different typologies of inactivation curves and the relative shape parameters p.

It is important to note that, simultaneously with Mafart *et al.* in 2002, also van Boekel presented a Weibull model, in the form of

$$log\left(\frac{N_t}{N_0}\right) = -\left(\frac{1}{2.303}\right)\left(\frac{t}{\alpha}\right)\beta$$

(8)

in which α and β are the scale parameter and the shape parameter, respectively (213).

1.6.2.3. Thermal tolerance at low a_w

Cross-protection to other environmental stressors is commonly observed after *Salmonella* is exposed to desiccation conditions. Increased thermal resistance following desiccation is one such example that has had a significant impact on the microbial safety of foods (3). This has implications on food processing as more intense heat treatment is required to ensure that the product is safe for consumption. Such treatments often result in a loss of product quality, stressing the importance of preventative measures as a critical food safety strategy.

It has been established that as a_w decreases, *Salmonella*'s thermal resistance increases (215-217). *Salmonella* Enteritidis inoculated in peanut butter prepared with a a_w of 0.2 had a *D*-value 7.05 minutes at 90°C versus 1.91 minutes in peanut butter prepared with a a_w of 0.8 at the same temperature (215). This notion was also observed in ground meat and bone meal where a 2- to 3-fold increase in the *D*-value was observed when dropping the starting moisture content from 15% to 10% and from 10% to 5% (217). In a study of *Salmonella* thermal tolerance when inoculated on almond kernels equilibrated to different water activities, the *D*-value at 68°C when the a_w was 0.946 was significantly shorter than the *D*-value at 70°C when the a_w was 0.601 (0.42 minutes and 15.15 minutes, respectively) (218). *Salmonella* inoculated on alfalfa seeds and thermally treated for 7 hours at 70°C had a greater reduction in cell count when the a_w of the seeds was 0.59 (1.01 Log (CFU g^{-1}) compared to 0.25

(0.38 Log CFU g^{-1}) (219). Previous work conducted in our laboratory also reported a similar trend as *D*-values of three *Salmonella* serovars (Agona, Typhimurium, Tennessee) were more than 20-fold smaller in cells exposed to a_w of 0.33 compared to those at 0.11 in toasted oat cereal (220).

The thermal resistance of *Salmonella* may also be influenced by the components present in the solution or the food matrix. It has been observed that, while the heat tolerance of eight strains of *Salmonella* increased as the a_w of different solutions of phosphate buffer decreased, addition of sucrose to lower the a_w resulted in greater protection than sorbitol, fructose, and glycerol (221). In food, this effect was observed in a comparative study of the thermal inactivation of *Salmonella* in peanut butter and all-purpose wheat flour. The *D*-values of *Salmonella* measured at 80°C were significantly different between peanut butter (17 min) and all-purpose wheat flour (6.9 min) (222). In that study, the authors suggested that the difference in thermal tolerance of *Salmonella* in the two matrices was due to the differences in a_w change during the thermal treatment. The potential effect of sucrose in toasted oat cereal was also evaluated by Chick (220). In that study, when *Salmonella* cells dried on cereal containing 25% sugar, slight changes in *D*- and δ- values were not consistent among serovars and water activity values.

In peanut butter, the a_w decreased significantly when the matrix was heated up from 20°C to 80°C, similarly to what observed in other fat rich products, such as peanut oil and oleic acid (223, 224). Decreases in a_w may be

protective for the bacterium, which may lead to higher thermal tolerance. Previously, a similar phenomenon was also observed for *B. cereus*, *C. botulinum*, and *C. sporogenes* by Ababouch and Busta (225). It is suggested that due to the elevated solubility at higher temperatures of the highly abundant nonpolar fats present in peanut butter, the water vapor pressure is decreased, as the interaction between water molecules and lipid molecules increases (226).

Another important factor to consider when analyzing the effectiveness of heat treatment is the presence of biofilms. Dhir and Dodd (227) demonstrated that *S.* Enteritidis cells attached to glass slides had a 2-fold greater *D*-value than planktonic cells. Interestingly, detached cells, i.e. cells that were originally in the form of biofilm and then detached from the polymeric matrix, exhibited the same increase in *D*-value than biofilm cells, suggesting that these cells maintained the characteristics of the biofilm phenotype during the heating process, at least in terms of thermal tolerance. Similarly, the ability of strains to form biofilm increased the tolerance to thermal process, as observed by Rojas *et al.* (228). In that study, six strains of *S*. Enteritidis, all capable of producing biofilm at different levels, were thermally treated after inoculation in wheat flour and equilibration to an a_w of 0.45. The researchers observed that *S*. Enteritidis biofilm formers had significantly higher *D*-values than non-biofilm formers (228).

1.6.3. Desiccation and thermal tolerance: a multi-stress response

In general, exposure to elevated temperatures in high moisture conditions causes protein denaturation, ribosomal damage, and enzyme deactivation (229,

230). In particular, destabilization of the 30S and 50S subunits of the ribosome through irreversible damage is the principal cause of bacterial inactivation (229, 231). One of the hypotheses to explain higher thermal tolerance in low a_w cells is that desiccation results in the loss of molecular mobility, which stabilizes ribosomal subunits (232). This is similar to what has been observed in spores, where high thermal tolerance is attributed to lower flexibility of the protein structures (233, 234).

It is important to remember that although dry conditions and low a_w can trigger protection to heat stress and damage, *Salmonella* still faces stress due to desiccation in low-moisture environments. Under these conditions, *Salmonella* activates cellular responses to prevent and minimize intracellular desiccation, and avoid membrane and protein damages due to water evaporation. *Salmonella* possesses numerous systems to respond to desiccation caused by the physical characteristics of many diverse environments such as soil, food, and abiotic surfaces (196, 235-238). Although the molecular and physiological mechanisms involved in the specific response are still uncertain, a study suggested that it may overlap with other common stress response systems, such as osmotic, thermal, and oxidative stress (239).

When water is lost, the concentration of solutes increases, determining an additional stress for the cell, which has to face a significant change in osmotic pressure and regulate the internal solute concentration to maintain the appropriate turgor (240-242). As a first defense, the cell activates a temporary

mechanism. During this response, the cell counterbalances the external high osmotic pressure accumulating potassium and its counter-ion glutamate inside the cytoplasm, to balance the accumulation of ions in the outside that drives water outside the cell. High levels of potassium glutamate in the cytoplasm, though, can impair enzyme activity. The cell then activates a second and long-term response and starts to accumulate other solutes, such as betaine, trehalose and proline (243).

Several studies have indicated that high osmolality can increase the tolerance of bacteria to high temperature as well as to oxidative stress (244-246). Exposure to NaCl was observed to increase thermal resistance of *Salmonella* at 50ºC, as well as tolerance to the oxidizing agent H_2O_2 (246). On the contrary, the presence of the osmoprotectant glycine betaine completely abolishes the high osmolality induced resistance (246), thus suggesting that the resistance mechanisms for both high temperature and oxidative stress are strictly linked to the activation of an osmotic response system. This theory is also suggested by a recent study in *Escherichia coli* (247), which found that genes usually involved in the oxidative-stress response system (*soxRS* and *oxyR*) were induced during exposure to high osmolality, high temperature, or a combination of both stresses, thus confirming an overlap of the two response systems. The study also reported that genes induced during the immediate response to high temperature were not the same induced during chronic exposure to temperature stress, suggesting that other stress response systems played a role in the cell survival strategy.

In *Salmonella*, the response to thermal shock entails two different responses (248-250). The first response is at the cytoplasmic level, where the *rpoH* mRNA is denatured due to elevated temperature. The denaturation allows for the attachment of the ribosome to the mRNA and the translation of the transcriptional factor σ^H (251, 252). This protein is responsible for the up-regulation of many genes, among them heat shock proteins that are mainly chaperones with the function of protecting the correct assembly and transport of proteins in the cell (250, 253, 254). A second mechanism of defense from heat shock involves the sigma factor σ^E, which acts on an extra-cytoplasmic level, on cell envelopes, protecting them from damages caused by heat and other stresses (248, 255-257).

The cell membrane plays an important role in the tolerance and adaptation to stress conditions. The membrane is subjected to important changes, since as water is lost from the bilayer, the polar heads of the lipids are forced closer, which increases the van der Waals interactions among the hydrocarbon acyl chains (258-260) This results in a higher level of packing of the lipids, which changes the membrane phase from Lα, i.e. the phase in which the fluidity of the membrane is greater thanks to more spacing among the lipid heads and in which the membrane is thinner (261, 262), to Lβ, a phase characterized by higher rigidity of the membrane due to a denser packing of the heads, and by increased thickness (261-263).

It has also been shown (264) that acid adapted cells of *Salmonella* Typhimurium had a higher thermal tolerance compared to non-adapted cells. This cross-protection mechanism, which was independent from the pH of the medium, was linked to the modification of the membrane composition. Acid-adapted cells, indeed, showed a lower unsaturated vs saturated fatty acid ratio in the membrane composition, which resulted in a less fluid membrane. Thermal inactivation studies with cells with this kind of adaptation resulted in higher *D*-values, suggesting that membrane composition could play a role in the overlapping response to acid and heat shock responses. The change in membrane composition as a reaction to changes in external conditions and as a way for temperature perception is an essential regulatory pathway for *Salmonella*, where the membrane structure and the lipid/protein composition are implicated in the transcriptional activation of heat shock genes (265).

The interplay between the different sigma factors to regulate *Salmonella* responses to stresses is extremely important. It has been demonstrated that σ^E controls expression of *rpoH* (266, 267). A recent study (268) showed that the antioxidant response promoted by σ^E and σ^H requires the starvation response sigma factor σ^S. That work showed that after a shift from nutrient rich medium to a nutrient poor medium, σ^E was activated. This activation led to an enhanced expression of *rpoH*, which was then responsible for the transcription of *hfq*, encoding for the RNA-binding protein HF-1. HF-1, in turn, promotes translation of σ^S, which results in an up-regulation of σ^S-dependent genes. Additional research

(269) previously demonstrated that a double mutation in σ^E and σ^S resulted in a decrease in *Salmonella* survival in stationary phase (less than 24 hours). It is clear that cellular response does not happen through the activation of a single cellular pathway, but that instead it is necessary an interplay among the different regulators in cells, at both the molecular and the cellular level.

1.7 Conclusions, rationale, and objectives

Microbial food contaminants represent a serious public health and economic issue. According to the CDC, non-typhoidal *Salmonella* spp. Are the first bacterial cause of foodborne illnesses in the US, and the first cause of hospitalization and deaths. Moreover, microbial contaminations of food products are reasons for costly food recalls, which eventually translate in an increase of costs for food companies and increased prices for consumers. While *Salmonella* was considered exclusively a dangerous contaminant of high moisture foods, in particular of animal origin, such as poultry, beef and eggs, this pathogen's outbreaks have been also associated with consumption of dry foods (nuts, peanut and nut butter, sesame paste, dry dog food). Low moisture foods (a_w < 0.6) have been considered safe since microbial growth is not supported at such a high level of dryness, but many dry foods have been reported to harbor *Salmonella*, for example chocolate and black pepper (270).

Many different studies have shown the ability of different serovars of this foodborne pathogen to survive in dry environments for very long periods, ranging

from weeks to months, and remain viable (191-193). It is now well-known that the initial exposure to desiccation and to the related stresses not only does not kill the microorganism, but is fundamental to trigger the development of thermal tolerance. This cross-protection mechanism has been abundantly demonstrated, and the interplay among different stress responses, such as osmotic, oxidative and heat stress, has been suggested to be the key to the development of the thermal tolerance. Despite the large amount of studies, though, the specific physiological and molecular processes involved and activated by the cell are still yet to be identified and clarified. A better knowledge of the systems involved in the response to desiccation and thermal tolerance, as well as a better understanding of their interplay, will be fundamental to identify effective combination of interventions to reduce *Salmonella*'s presence in foods.

The goal of this work was to determine physiological and molecular mechanisms that contribute to *Salmonella*'s ability to survive desiccation and develop thermal tolerance. The central hypothesis was that the survival and persistence of *Salmonella* in dry conditions and the subsequent cross-protection to high temperatures rely on molecular and physiological mechanisms. These mechanisms are common to multiple stress response systems, e.g. oxidative, heat, starvation, and osmotic stress. Therefore, exposure to desiccation and thermal treatment induces similar modifications in the gene expression profile, protein profile, regulatory, as well as biosynthetic, pathways, and cell composition.

Null Hypothesis I:

The transcriptome of *S. enterica* does not change after adaptation to desiccation and low a_w.

Null Hypothesis II:

The ability of *S. enterica* to withstand desiccation and low a_w is not affected when the putative virulence genes are knocked-out and rendered non-functional.

Null Hypothesis III:

Desiccation, low a_w, and thermal treatment do not have any effect on the cell morphology of *S. enterica.*

Null Hypothesis IV:

Growth conditions prior to desiccation and matrices do not influence desiccation survival and development of thermal tolerance by *S. enterica.*

Null Hypothesis V:

The proteome of *S. enterica* does not change after adaptation to desiccation, low a_w, and thermal treatment.

The main objectives of this work were to:

1. Identify genes involved in desiccation resistance development in *S. enterica* serovar Typhimurium;
2. Determine changes in cell morphology due to desiccation, prolonged exposure to low a_w, and thermal treatment;

3. Assess the effect of matrices and growth conditions on desiccation survival and thermal tolerance of *S. enterica* serovar Typhimurium;

4. Identify proteins involved in the resistance to desiccation and thermal tolerance in *S. enterica* serovar Typhimurium.

2. General Response of *Salmonella enterica* serovar Typhimurium to Desiccation: a New Role for the Virulence Factors *sopD* and *sseD* in Survival

2.1. Summary

Salmonella can survive for long periods under extreme desiccation conditions. This stress tolerance poses a risk for food safety, but relatively little is known about the molecular and cellular regulation of this adaptation mechanism. To determine the genetic components involved in *Salmonella*'s cellular response to desiccation, we performed a global transcriptomic analysis comparing *S. enterica* serovar Typhimurium cells equilibrated to low water activity (a_w 0.11) and cells equilibrated to high water activity (a_w 1.0). The analysis revealed that 719 genes were differentially regulated between the two conditions, of which 290 genes were up-regulated at a_w 0.11. Most of these genes were involved in metabolic pathways, transporter regulation, DNA replication/repair, transcription and translation, and, more importantly, virulence genes. Among these, we decided to focus on the role of *sopD* and *sseD*. Deletion mutants were created and their ability to survive desiccation and exposure to a_w 0.11 was compared to the wild-type strain and to an *E. coli* O157:H7 strain. The *sopD* and *sseD* mutants exhibited significant cell viability reductions of 2.5 and 1.3 Log (CFU/g), respectively, compared to the wild-type

after desiccation for 4 days on glass beads. Additional viability differences of the mutants were observed after exposure to a_w 0.11 for 7 days. *E. coli* O157:H7 lost viability similarly to the mutants. Scanning electron microscopy showed that both mutants displayed a different morphology compared to the wild-type and differences in production of the extracellular matrix under the same conditions. These findings suggested that *sopD* and *sseD* are required for *Salmonella*'s survival during desiccation.

2.2. Introduction

Salmonella enterica, a Gram negative bacterium belonging to the Enterobacteriaceae family, is a foodborne human pathogen that can cycle from the environment to animals and humans through their fecal matter (271-273). Because of the wide variety of environments *Salmonella* can be exposed to, it can adapt to very diverse physical or chemical conditions. Generally, one of the most important factors impacting the ability of an organism to survive in a certain environment is the presence and, more importantly, the availability of water for chemical and biological reactions, a concept defined as water activity (a_w). a_w is expressed as the ratio between the vapor pressure of water with a solute and the vapor pressure of pure water.

A relatively high a_w is essential for microbial growth, since at low a_w enzymatic reactions are inhibited and metabolism is reduced (274, 275). Gram negative bacteria such as *E. coli*, *Salmonella* and *Vibrio* require an a_w greater

than 0.95 to grow (276). As a result, one of the main strategies to control bacterial proliferation in food matrices is the reduction of a_w to create a low moisture environment (275, 277). Most Enterobateriaceae are well adapted to persist in dry environmental conditions and *Salmonella* is no exception. To survive under harsh conditions, such as those found in a dry environment, bacteria need to activate a variety of cellular stress responses.

One of the first protection mechanisms activated by the shift from humid to dry environments is the response to osmotic stress induced by the decrease of water due to evaporation and the relative increase of the solute. In particular, this process makes the environment increasingly hypertonic, thus triggering specific molecular mechanisms that allow the cell to regulate the internal solute concentration to maintain the appropriate turgor (183, 217, 278). Indeed, when exposed to low a_w, *Salmonella* prevents and minimizes the loss of intracellular water and avoids membrane and protein damage due to the progressively hypertonic environment by increasing the influx of osmoprotectants. A recent study reported that up-regulation of osmoprotectant genes and operons such as *48rop*, *proVWX*, and *osmU* can be observed after short-term desiccation on stainless steel coupons (279). Up-regulation of some of these same genes was also observed after 2 h exposure to a_w 0.11 (280).

As aforementioned, osmotic protection is only one of the first mechanisms deployed by the cell in response to desiccation. A recent study from Gruzdev et *al.* suggested that it is likely to be part of a network of stress responses, such as

oxidative and thermal stress, that act in a concerted fashion and modulate each other (86). In fact, genes involved in the oxidative stress response through the formation and/or protection of iron-sulfur (Fe-S) clusters, such as *nifU*, *nifS*, *iscA*, part of the nitrogen fixation system (NIF), and *sufD* (281-285), have been found up-regulated after desiccation of *Salmonella* on Petri dishes (286). In the same study, *fnr*, encoding for the fumarate and nitrate reduction protein and one of the master regulators for the metabolic shift from aerobic to anaerobic conditions, was also induced following desiccation. The knockout mutant Δ*fnr* showed impaired ability to survive dehydration and long-term storage at room temperature (286).

Interestingly, pre-exposure to desiccation has also been shown to induce protection against heat treatment (287-289). Most of the theories are based on physicochemical properties of the cell and focus on the stabilization of proteins during thermal exposure when less water is present (197, 290, 291). However, some reports indicated that *Salmonella* thermal tolerance persists for a short period of time after rehydration (239). Additionally, non-typhoidal *Salmonella* spp. And STEC *E. coli* showed higher tolerance to desiccation than non-pathogenic *E. coli* (197), although these species share almost identical physicochemical properties. These observations suggest that the thermal tolerance is not limited to chemical and physical phenomena, but could be in part a consequence of the complex network of overlapping stress responses induced by desiccation.

In addition to heat, exposure to desiccation has been linked with cross-protection for a multitude of other stressors in *Salmonella*, such as sodium hypochlorite, sodium chloride, bile salts, and hydrogen peroxide (239). The ability of *Salmonella* to overcome different stresses is crucial for its virulence, since it is an essential capability during the infection process. To colonize the host, *Salmonella* has to survive extra- and intra-cellularly in the stomach and the intestine, where it is exposed to acid, osmotic, and oxidative stress, as well as starvation. Induction of virulence genes *hilA*, *invA*, and *spiC* was found after drying and storage in dry milk for short periods in *Salmonella* cells, both in planktonic and biofilm state (292).

The production of curli, thin aggregative fimbriae, cellulose, and lipopolysaccharides has also been reported to be important for survival under dry conditions (280, 293-295). One of the hypotheses is that due to their high water retention quality, exopolysaccharides work as a water deposit (295). Mutants in lipolysaccharides have been shown to be more sensitive to desiccation than parental *Salmonella* strains (295). *Salmonella*'s ability to produce biofilm has also been reported to be important for desiccation survival on polypropylene discs (296).

In this work, we identify genetic components that are involved in the ability of *Salmonella*'s cells to survive reduction in moisture and exposure to very low water activity. In particular, two virulence genes, *sopD* and *sseD*, that are important for its survival to desiccation.

2.3. Materials and methods

2.3.1. Bacterial strains and culture preparation

The strains used in this study included *Salmonella enterica* serovar Typhimurium strain ATCC 14028 (from now on *S. enterica* serovar Typhimurium), *Escherichia coli* O157:H7 strain ATCC 43895 (from now on *E. coli* O157:H7), and two deletion mutants of the strain *S. enterica* serovar Typhimurium, Δ*sopD* and Δ*sseD*, obtained as described below by *camR* and *aphA-2* insertions, respectively. The stock cultures were stored at -55°C in a 5:1 solution of Luria-Bertani broth (LB; BBL, Detroit, MI) and glycerol. Working cultures were prepared in tryptic soy broth (TSB, Neogen, Inc., Lansing, MI) or 0.01 M glucose-supplemented LB broth (LBglc) from frozen stock cultures and grown overnight at 37°C shaking at 250 rpm. For mutant strain working cultures, chloramphenicol and kanamycin were added to LBglc to a final concentration of 50 and 100 µg/mL, respectively.

2.3.2. Inoculation on filters and RNA extraction

Working cultures of *S. enterica* serovar Typhimurium were freshly inoculated in TSB and grown for 3 h at 37°C shaking at 250 rpm. The cultures were collected through centrifugation (10 min at 4,696 x g) and washed twice with distilled sterile water (DSW) to eliminate nutrient residues. Approximately 10^9 CFU were spotted on 0.2 µm polycarbonate filters (Merck Millipore Ltd., Billerica, MA) and allowed to air-dry for 24 h at room temperature in a biosafety cabinet. Filters were placed in desiccators containing water or a saturated

solution of lithium chloride 99% (Acros Organics, Thermo Fisher Scientific, Waltham, MA) to allow equilibration to a_w 1.0 and 0.11 respectively. Separate samples of corn starch were included as controls to monitor the a_w of the desiccators. After 4 days in the desiccators at 25°C, the a_w of corn starch samples were measured and the total RNA was extracted from *Salmonella* cells using the RNAprotect Bacteria Reagent and Rneasy Mini Kit (Qiagen, Hilden, Germany). The experiments were repeated three times on different days. Each time, three technical replicates were performed. The total RNA was extracted individually for each replicate and then the RNA was pooled together from all the replicates for the same conditions.

2.3.3. RNA Seq and global transcriptional analysis

Total RNA was processed at the University of Minnesota Genomic Center. Briefly, samples were quantified using a fluorimetric RiboGreen assay. Total RNA integrity was assessed using capillary electrophoresis with 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA), generating a RNA Integrity Number (RIN). Samples were converted to Illumina sequencing libraries using the TruSeq RNA Sample Preparation and Library Preparation Kit (Illumina Inc., San Diego, CA). In summary, 1 µg of total RNA was reverse transcribed into cDNA. The cDNA was fragmented, blunt-ended, ligated to barcoded adaptors, and amplified using 15 cycles of PCR. Final library size distribution was validated using capillary electrophoresis and quantified using fluorimetry (PicoGreen) and via Q-PCR. Indexed TruSeq libraries were then normalized, pooled, hybridized to a

paired end flow cell, and individual fragments were clonally amplified by bridge amplification on the Illumina cBot (Illumina Inc., San Diego, CA). Once clustering was completed, the flow cell was loaded on the HiSeq 2000 (Illumina Inc., San Diego, CA). Sequencing was performed on both strands.

Base call files for each cycle of sequencing were generated by Real-Time Analysis software (Illumina Inc., San Diego, CA). Primary analysis and de-multiplexing were performed using CASAVA software v1.8.2 (Illumina Inc., San Diego, CA). The end results of the CASAVA workflow, de-multiplexed FASTQ files, were analyzed using the DNASTAR SeqManNgen and ArrayStar softwares (DNASTAR, Inc., Madison, WI). The reads were assembled and mapped to the genome using SeqManNgen, while the levels of expression were estimated using ArrayStar.

The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (297) and are accessible through GEO Series accession number GSE86580 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE86580).

2.3.4. Construction of deletion mutants

Two mutant strains were generated using the λ Red-mediated homologous recombination (298). The plasmid pKD46 was introduced into *S. enterica* serovar Typhimurium using a MicroPulser electroporator (Bio-Rad Labs., Hercules, CA) at 1.7 kV. Primers used for knockout of *sopD* and *sseD* genes are reported in Table 2.1. All knockout primers were 60 nucleotides long, with 39

nucleotides homologous to the upstream or downstream regions flanking the targeted gene, and 21 nucleotides homologous to a universal cap designed in the drug resistance cassette (kindly provided by Dr. Roth, University of California at Davis, Davis, CA). The procedure used to make donor DNA fragments followed the protocol previously described (299). Briefly, the PCR amplification protocol was: 95°C for 5 min; 95°C for 1 min, 55°C for 1 min, 72°C for 1:40 min × 30 cycles, and 72°C for 5 min. The 55°C melting temperature of our fragments was calculated on the 21-nucleotide caps.

Table 2.1. Primers used for λ Red-mediated recombination.

Gene	Primer ID	Sequence (5'-3')[a]
sopD	*sopD*-F	CGGATATTGAATAATATAAATTTGAAGGAAAATATTATG**CACACAACCACACCACACCAC**
	sopD-R	TTATATTACTGACTATCTTTATGTCAGTAATATATTACG**CACCAAACACCCCCCAAAACC**
sseD	*sseD*-F	ATAGCTGGCTATCGCGCTTAATCTGAGGATAAAAATATG**CACACAACCACACCACACCAC**
	sseD-R	CTATTTCTTGCACCATGTTTACCTCGTTAATGCCCGGAG**CACCAAACACCCCCCAAAACC**

[a]Nucleotides homologous to the universal caps are in bold.

The *sopD* and *sseD* coding sequences were disrupted with the chloramphenicol (*camR*) and the kanamycin (*aphA-2*) resistance cassette, respectively, leaving the ATG region intact. The resistance cassettes were inserted in 3'-5' to avoid polar effects of the universal cassette promoter on the downstream genes (Appendix 1).

The deletion of the *sopD* and *sseD* genes by substitution and insertion of the antibiotic cassette was verified by PCR amplification (Appendix 1) and Sanger sequencing. Primers used are listed in Appendix 2. The sequenced reads were matched against NCBI database using the BLAST function (300) for the antibiotic cassette portion, while the gene's upstream and downstream regions were identified using Artemis platform (Wellcome Trust Sanger Institute) (301). The total genome of the two mutants was also extracted using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO) and sequenced on a HiSeq 2500 sequencer (Illumina, San Diego, CA). The assembling and mapping results were obtained using the DNASTAR SeqManNgen software (DNASTAR, Inc., Madison, WI).

2.3.5. Growth curve

The growth rates and generation times were determined using the optical density (OD) measured at 600 nm with the Epoch 2 microplate reader (Bio Tek Instruments, Inc., Winooski, VT). Liquid cultures in LBglc were incubated overnight at 37°C with shaking at 250 rpm. The OD of each strain was then adjusted to 0.02 in LBglc and 200 µL aliquots were pipetted to 96-well plates. The plates were transferred to the plate reader with an incubation temperature of 37°C and orbital shaking. The Ods were recorded every 10 minutes for 24 h. Growth rates during the exponential phase were calculated by a regression of ln(OD) vs. time, where the slope was the growth rate based on the Monod

equation (dN/dt = μN, where N is cell concentration expressed as OD, t is time, and μ is growth rate).

2.3.6. Viability experiments on micro glass beads

Bacterial cultures were grown overnight at 37°C with shaking at 250 rpm in LBglc. The cultures were collected through centrifugation (10 min at 4,696 x g) and washed twice with DSW to eliminate nutrient residues. For these experiments, we decided to use glass beads (150-250 μm) (Corpuscular Inc., Cold Spring, NY). Our decision was motivated by the need to increase the number of cells, while still ensuring that the cells were evenly distributed and exposed to low a_w. The greater total surface area offered by the beads compared to the filters allowed for a larger number of cells and for the formation of a thinner layer of adhering cells (as confirmed by the SEM micrographs). The washed pellets were re-suspended in 10 mL DSW and used to inoculate 10 g of sterile glass beads. Inoculated glass beads were spread on a sterile Petri dish and dried for 4 days at 38.5 ± 0.5˚C. For viable cell enumeration, 100 μL of each re-suspension were serially diluted in sterile saline (NaCl 0.9%), and 100 μL were spread plated on differential tryptic soy agar (dTSA) [TSA (Neogen, Inc.) supplemented with ammonium iron (III) citrate 16% (Fluka Analytical, Sigma-Aldrich, St. Louis, MO) (0.8 g/L) and sodium thiosulfate 98.5% (Acros Organics, Thermo Fisher Scientific, Waltham, MA) (6.8 g/L)].

After drying, the beads were distributed into sterile 200 μL PCR plastic tubes (Thermo Fisher Scientific). For exposure to a_w 0.11 and 1.0, the samples

were equilibrated for 7 days at 25˚C in desiccators containing a saturated lithium chloride (Acros Organics, Thermo Fisher Scientific) solution or sterile distilled water (SDW). After 7 days, the water activity of samples was measured (cutoff value: a_w reference ± 0.02), and the samples were sealed. To determine the survival rate, beads for every sample were serially diluted in saline and spread plated on dTSA for cell enumeration. The recovery after every treatment was measured as cell viability in Log (CFU/g), and the survival rate was calculated as viability change in Log (CFU/g).

2.3.7. Scanning electron microscopy

Samples were collected after inoculation on glass beads, dried for 4 days, and equilibrated 7 days to a_w 0.11 and 1.0. Immediately after collection, samples were fixed with a solution of 1% paraformaldehyde, 1% glutaraldehyde, and 0.05 M sodium cacodylate as previously described (302). The samples were fixed overnight and then dehydrated through an ethanol series (10, 25, 70, 90, 95, and 100% ×2 for 24 h each) and HMDS series (30, 60, and 100% ×2 for 20 min each). Samples were transferred into 100% HDMS, air-dried for 48 h at room temperature, placed on 9.5 mm aluminum stubs with adhesive carbon tape, and coated with 20 nm of gold-palladium using the SC7620 Mini Sputter Coater (Quorum Technologies, Inc, Guelph, Canada). Scanning electron microscopy (SEM) imaging was performed with the JSM 6060LV scanning electron microscope (JEOL USA, Inc., Peabody, MA) using a 15 kV accelerating voltage.

2.3.8. Statistics

Generation times for the wild-type and mutant strains were calculated averaging three independent biological replicates (n = 3). The generation time for each replicate was determined using the average OD of four technical replicates. All the viability experiments on glass beads were repeated at least four times ($n \geq 4$) in different days (biological replicates). Each biological replicate consisted of three technical replicates. Technical replicate results were averaged to obtain the Log (CFU/g) for each biological replicate.

Significance, expressed as p-value, was calculated using a two-tailed Student's t-test assuming equal variance for all experiments. Threshold for significance was set at $p \leq 0.05$. Standard error of the mean (SE) was used to calculate the variation among samples. Averages, p-values, and SE were performed on the results of the biological replicates for each strain at each condition.

2.4. Results

2.4.1. Global transcriptional analysis

The transcriptional profile of *S. enterica* serovar Typhimurium cells air-dried and equilibrated to a_w 0.11 was compared to that of cells dried and equilibrated to a_w 1.0. Out of 4,489 genes (303) 719 (16%) were differentially expressed between the two conditions. Among these, 290 genes (40.3%) were up-regulated (2-fold cutoff) (Appendix 3). We decided to focus on the up-

regulated genes because they are those most likely necessary for the adaptation to low water activity. These genes were categorized based on the KEGG Orthology (KO) database (304). We found 5 functional classes: 1) metabolism (52 genes); 2) genetic information processing (24 genes); 3) environmental information processing (25 genes); 4) cellular processes (3 genes), and 5) infectious diseases (2 genes, both classified as virulence factors). The remaining 184 genes did not belong to any orthology group and were, therefore, unclassified. Table 2.2 lists a selected group of up-regulated genes and their functions by KO categories and sub-categories.

Table 2.2. Selected up-regulated genes in *Salmonella* exposed to a_w 0.11 versus 1.0.

	Locus	Gene name	Function	Fold change
Ribosomal				
	STM0095	*rluA*	23S rRNA/tRNA pseudouridine synthase A	4.45
	STM3441	*rpsJ*	30S ribosomal protein S10	3.42
	STM1835	*rrmA*	23S rRNA methyltransferase A	2.05
Transporters				
	STM0006	*yaaJ*	alanine/glycine transport protein	5.48
	STM3986	*trkH*	potassium transporter	3.70
	STM1491	*osmV*	proline/glycine betaine transport systems	2.91
	STM1379	*orf48*	amino acid permease	2.51
	STM2353	*hisQ*	histidine/lysine/arginine/ornithine transport protein	2.37

	STM1893	*znuB*	high-affinity zinc transporter membrane protein	2.28
	STM1806	*nhaB*	sodium/proton antiporter	2.28
	STM0835	STM0835	manganese transport transcriptional regulator MntR	2.05
	STM0568	*pheP*	phenylalanine transporter	2.05
tRNAs				
	STM3933	*leuT*	tRNA-Leucine	12.32
	STM3890	*gltU*	tRNA-Glutamate	10.50
	STM3932	*hisR*	tRNA-Histidine	5.48
	STM2394	*argW*	tRNA-Arginine	3.42
	STM4554	*leuP*	tRNA-Leucine	3.42
	STM0254	*aspU*	tRNA-Aspartate	3.19
	STM1134	*serX*	tRNA-Serine	3.08
	STM2824	STM2824	tRNA-Arg	2.05
	STM0674	*glnV*	tRNA-Gln	2.05
	STM4178	*gltV*	tRNA-Glu	2.05
	STM3037	*glyU*	tRNA-Gly	2.05
	STM2989	*metZ*	tRNA-Met	2.05
	STM4143	*tyrU*	tRNA-Tyr	2.05
Transcription/ translation regulators				
	STM2836	*gutM*	DNA-binding transcriptional activator GutM	4.79
	STM1523	*yneJ*	transcriptional regulator	4.11
	STM0859	STM0859	transcriptional regulator	4.11
	STM1549	STM154	translation initiation inhibitor	3.42

		9		
	STM2794	*ygaE*	DNA-binding transcriptional regulator CsiR	3.42
	STM4511	*yjiE*	DNA-binding transcriptional regulator	3.42
	STM3681	STM3681	transcriptional regulator	2.46
	STM1547	STM1547	transcriptional regulator	2.40
	STM1706	*yciH*	translation initiation factor Sui1	2.40
	STM3523	*glpR*	DNA-binding transcriptional repressor GlpR	2.33
	STM1773	*ychA*	transcriptional regulator	2.05
	STM3667	*yiaJ*	transcriptional repressor	2.05
	STM1488	*mlc*	pts operon transcriptional repressor	2.05
DNA replication and repair				
	STM0646	*holA*	DNA polymerase III subunit delta	4.11
	STM0263	*rnhA*	ribonuclease H	4.11
	STM0821	*dinG*	ATP-dependent DNA helicase DinG	3.73
	STM1821	*yoaA*	DNA helicase	3.42
	STM2496	*yfgE*	DNA replication initiation factor	3.42
	STM1201	*holB*	DNA polymerase III subunit delta'	2.74
	STM2223	*yejH*	ATP-dependent helicase	2.74
	STM1898	*ruvC*	Holliday junction resolvase	2.05
	STM0481	*priC*	primosomal replication protein N"	2.05
Fimbriae				
	STM4593	*sthB*	fimbrial usher protein	5.48
	STM1974	*fliK*	flagellar hook-length control protein	3.94

	STM0023	*bcfC*	fimbrial usher	3.42
	STM1913	*flhA*	flagellar biosynthesis protein FlhA	3.42
	STM1973	*fliJ*	flagellar biosynthesis chaperone	3.42
	STM4591	*sthE*	major fimbrial subunit	3.42
	STM0177	*stiA*	fimbrial subunit	2.05
	STM4594	*sthA*	fimbrial chaperone	2.05
Virulence				
	STM1399	*sscA*	secretion system chaperone	12.32
	STM1397	*sseA*	secretion system chaperone protein	3.70
	STM2945	*sopD*	secreted effector protein	3.13
	STM1170	*mviN*	virulence protein	3.03
	STM3764	*mgtC*	Mg2+ transport protein	2.40
	STM1401	*sseD*	translocation machinery component	2.05
Membrane				
	STM3178	*ygiY*	sensor protein QseC	3.42
	STM3372	*mreD*	rod shape-determining protein MreD	2.57
	STM3373	*mreC*	rod shape-determining protein MreC	2.22
	STM3374	*mreB*	rod shape-determining protein MreB	2.05

More specifically, multiple amino acid transporters where found up-regulated, including the alanine/glycine transporter *yaaJ*, the histidine/lysine/arginine/ornithine transporter *hisQ*, and the phenylalanine transporter *pheP*. Other genes involved with ion transportation were also found induced by low water activity: for example, *trkH* for potassium, *znuB* for zinc,

nhaB for sodium, and also the transcriptional regulator *mntR* for manganese. Several tRNA species were up-regulated: the tRNA for leucine (*leuP* and *leuT*), glutamate (*gltU*), histidine (*hisR*), arginine (*argW*), aspartate (*aspU*) and serine (*serX*) were the most abundant in low water activity. DNA replication genes, such as *holA*, *rnhA*, *dinG*, and DNA repair genes (*ruvC*, *priC*), were also induced in low water activity conditions.

Other up-regulated gene groups included transcriptional and translational regulators (i.e. *gutM*, *yneJ*, *yciH*) and ribosomal genes (i.e. *rluA*, *rpsJ*, *rrmA*). Genes involved in fimbriae and flagella biosynthesis were also up-regulated (i.e. *flhA*, *sthE*, *stiA*). Although we detected only two virulence genes by KO classification, *sopD* and *sseD*, four other known virulence factors were up-regulated in low water activity conditions. These virulence factors included *sscA*, a chaperon for the SseC translocon (305); *sseA*, a class II chaperone specific for translocon proteins SseB and SseD (306-308); *mviN*, involved in peptidoglycan biosynthesis and required for virulence in mice (309, 310); and *mgtC*, part of the *mgtBC* operon in SPI-3, involved in the regulation of the membrane potential (311), and transcriptionally controlled by PhoP/PhoQ system (312).

2.4.2. Δ*sopD* and Δ*sseD* mutant verification and sequencing

Based on the transcriptomic analysis results, we generated mutants in two virulence factors, *sopD* and *sseD*. Disruption of *sopD* by insertion of the chloramphenicol resistance cassette (*camR*) and of *sseD* by the kanamycin resistance cassette (*aphA-2*) was confirmed by PCR and sequencing. Genome-

wide sequencing of the two mutants confirmed that the only variants present were localized in the targeted genes. When grown in LBglc at 37°C with aeration, both the mutants had the same generation time (G) compared to the wild-type (WT) strain: 22 min (SE 0.4 min) for WT, 21 min (SE 0.3 min) for Δ*sopD* (p = 0.33) and 21 min (SE 0.1 min) for Δ*sseD* (p = 0.11).

2.4.3. Survival on glass beads

To test the effect of *sopD* and *sseD* genes in response to desiccation, *S. enterica* serovar Typhimurium wild-type (WT), mutant strains, and *E. coli* O157:H7 were inoculated on micro glass beads, dried, and equilibrated to a_w 0.11 and 1.0 (Figs 2.1 and 2.2). As mentioned in the Materials and Methods, the use of glass beads was chosen to allow for a larger starting inoculum, while ensuring a homogenous surface distribution of the cells.

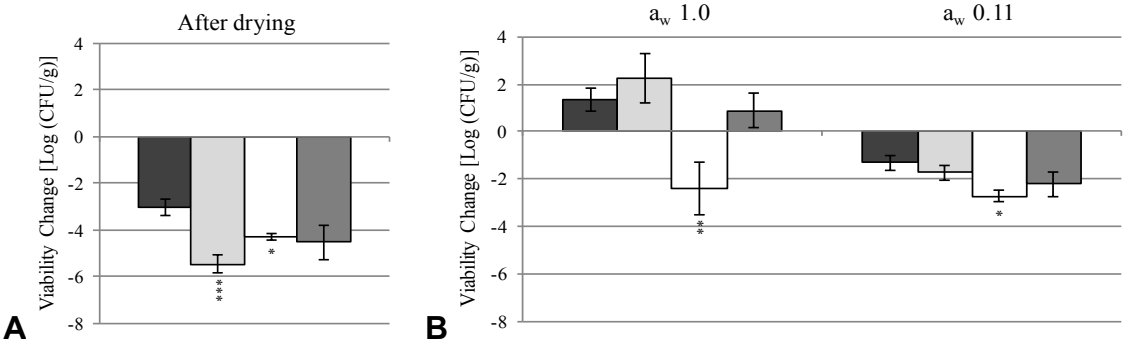


Fig 2.1. Changes in cell viability during drying and equilibration on glass beads. Changes in cell viability [Log (CFU/g)] of *S. enterica* serovar Typhimurium wild-type (WT; dark grey), Δ*sopD* (light grey), Δ*sseD* (white), and *E. coli* O157:H7 (grey) after 4 days drying (A) and after 7 days of equilibration to

a_w 0.11 and 1.0 (B). Bars indicate standard error of the mean (SE). Stars indicate *p*-values < 0.05 (*), < 0.01 (**), and < 0.005 (***) in comparison with *Salmonella* WT under the same condition.

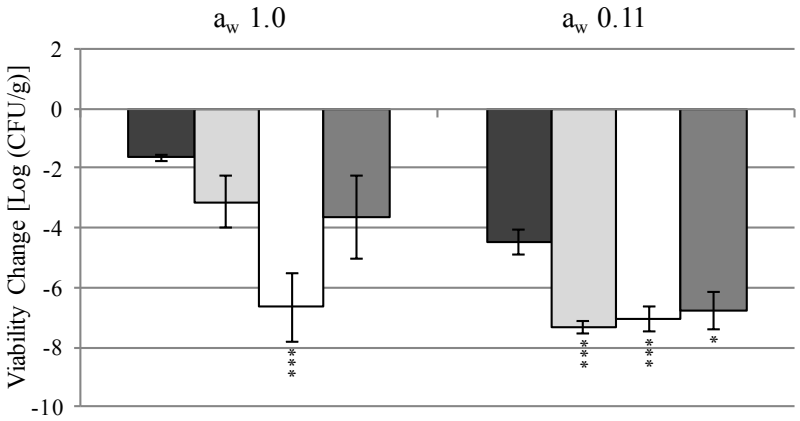


Fig 2.2. Total changes in cell viability during drying and equilibration on glass beads. Total changes in cell viability [Log (CFU/g)] of *S. enterica* serovar Typhimurium wild-type (WT, dark grey), Δ*sopD* (light grey), Δ*sseD* (white), and *E. coli* O157:H7 (grey) after 11 days of treatment (4 days drying and 7 days of equilibration to a_w 1.0 or 0.11). Bars indicate standard error of the mean (SE). Stars indicate *p*-values < 0.05 (*), < 0.01 (**) and < 0.005 (***) in comparison with *Salmonella* WT under the same condition.

Cell counts decreased for all the strains after drying (Fig 2.1 A) [*Salmonella* WT, -3.0 Log (CFU/g); Δ*sopD,* -5.5 Log (CFU/g); Δ*sseD*, -4.3 Log (CFU/g); and *E. coli* O157:H7, -4.5 Log (CFU/g)]. The differences between *Salmonella* WT and the mutants were significant (*p*-values 0.0003 for Δ*sopD* and

0.02 for Δ*sseD*). Although the *E. coli* O157:H7 count decreased, it was not significantly different from *Salmonella* WT (p = 0.06).

After equilibration to a_w 1.0 (Fig 2.1 B), the cell counts for all the strains except Δ*sseD* increased compared to drying [*Salmonella* WT, 1.3 Log (CFU/g); Δ*sopD*, 2.3 Log (CFU/g); and *E. coli* O157:H7, 0.9 Log (CFU/g)]. Conversely, Δ*sseD* decreased by 2.4 Log (CFU/g). This difference was statistically significant when compared to the WT and Δ*sopD*, but not to *E. coli* O157:H7 (p-values 0.007, 0.014, and 0.06, respectively).

After equilibration to a_w 0.11 (Fig 2.1 B), the cell counts for all the strains decreased compared to drying [*Salmonella* WT, -1.3 Log (CFU/g); Δ*sopD*, -1.7 Log (CFU/g); Δ*sseD*, -2.7 Log (CFU/g); and *E. coli* O157:H7, -2.2 Log (CFU/g)], although only Δ*sseD* had a significantly lower recovery than *Salmonella* WT (p = 0.012).

The large decrease in cell count of Δ*sopD* during drying lowered the cell count close to the detection limit of the experiment [2.7 Log (CFU/g)]. Therefore, it was difficult to estimate the additional decrease in viability and its significance after exposure to a_w 0.11. For this reason, we also calculated the total change in cell count for both a_w over the entire treatment period (11 days, Fig 2.2). At a_w 1.0, the total change in cell counts after the 11 day treatment was not significantly different between *Salmonella* WT, Δ*sopD*, and *E. coli* O157:H7 [*Salmonella* WT, -1.6 Log (CFU/g); Δ*sopD*, -3.1 Log (CFU/g); and *E. coli* O157:H7, -3.7 Log (CFU/g)]. On the contrary, Δ*sseD* had an overall reduction of

6.7 Log (CFU/g), significantly larger than *Salmonella* WT and Δ*sopD*, but not *E. coli* O157:H7 (*p*-values 0.0004, 0.03, and 0.14, respectively). At a_w 0.11 (Fig 2.2), we observed large total reductions in cell counts [*Salmonella* WT, -4.5 Log (CFU/g); Δ*sopD*, -7.4 Log (CFU/g); Δ*sseD*, -7.1 Log (CFU/g); and *E. coli* O157:H7, -6.8 Log (CFU/g)]. The mutants and *E. coli* O157:H7 had significantly lower cell viability compared to *Salmonella* WT (*p*-values 0.0001 for Δ*sopD*, 0.003 for Δ*sseD*, and 0.01 for *E. coli* O157:H7).

2.4.4. Scanning electron microscopy of *S. enterica* serovar Typhimurium wild-type, Δ*sopD*, Δ*sseD*, and *E. coli* O157:H7

Observations by SEM of *S. enterica* serovar Typhimurium wild-type (WT), Δ*sopD*, Δ*sseD*, and *E. coli* O157:H7 cells on glass beads showed WT and *E. coli* O157:H7 cells as rod-shaped cells with an average length of 1.5 μm and an average width of 0.5 μm. In contrast, both mutants displayed different cell morphology, more coccobacillary, with markedly smaller, rounder, and shorter cells, 1 μm in length or less (Fig 2.3).

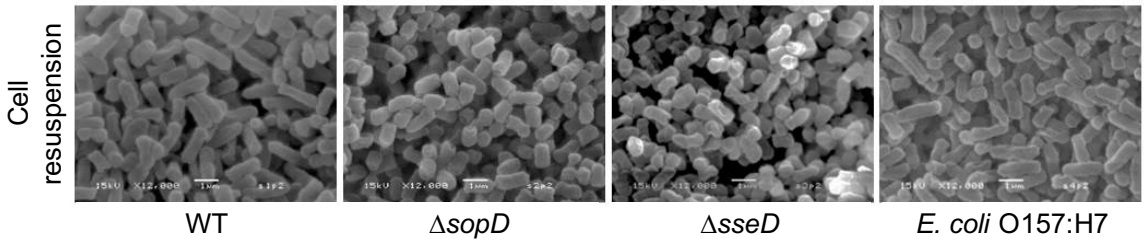


Fig 2.3. Scanning electron microscopy images of *S. enterica* serovar Typhimurium wild-type (WT), Δ*sopD* and Δ*sseD* strains, and *E. coli*

O157:H7. Cells were collected from the overnight cell re-suspension used to inoculate the glass beads. The images show the change in cell morphology for the two mutants. Magnification and scale bar are embedded in the images.

After drying, WT cells still appeared rod-shaped, although some cells displayed cell surface corrugations indicating a loss of turgidity (Fig 2.4). Cells were also embedded in a thick extracellular matrix. After drying, both mutants maintained a smaller size than the WT with a spheroidal shape and an evident indentation in the middle of the cell. Additionally, the cell density on the glass bead surface was lower than what was observed in the parental strain. Although Δ*sopD* also produced an extracellular matrix, it lacked the three-dimensional structure observed for the WT. The matrix did not embed the cells but was attached to the bead surface. The matrix also had characteristic cell-shaped discontinuities where cells detached. In the same conditions, Δ*sseD* did not produce a homogeneous matrix, but presented an intricate network of ropy filaments. *E. coli* O157:H7 cells had morphology similar to *Salmonella* WT, but they produced a matrix similar to Δ*sopD*.

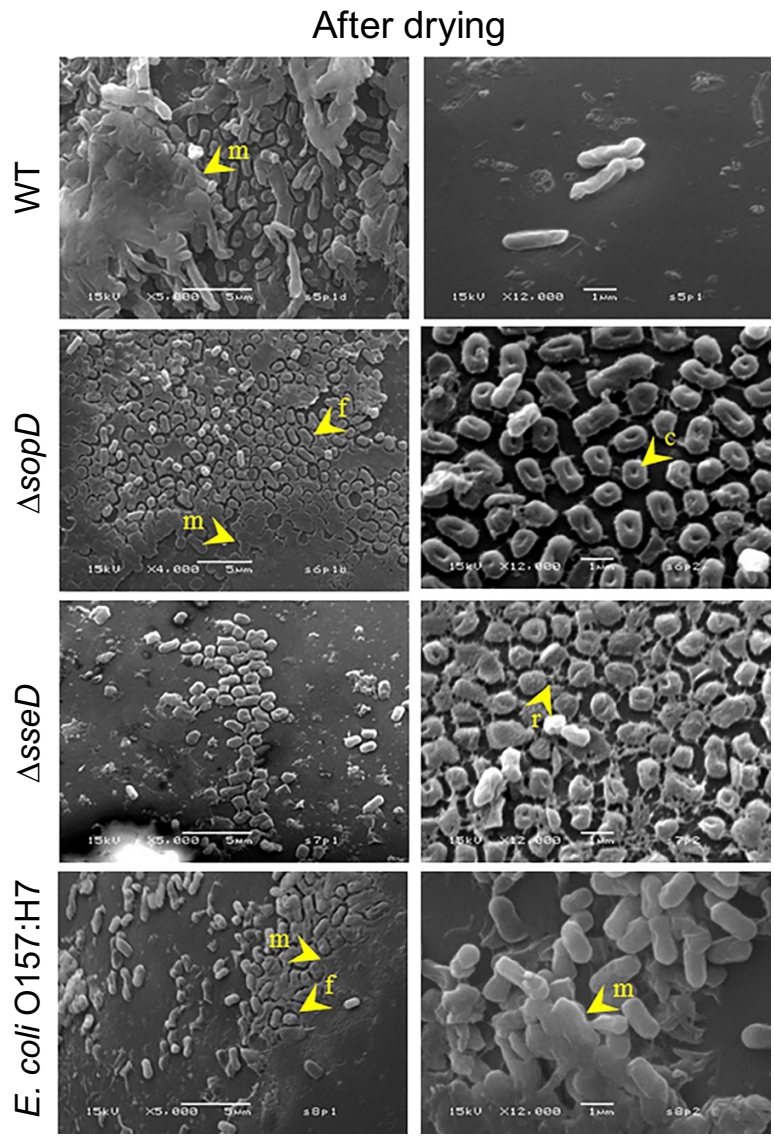


Fig 2.4. Scanning electron microscopy images of *S. enterica* serovar Typhimurium wild-type (WT), Δ*sopD* and Δ*sseD* strains, and *E. coli* O157:H7 after drying. Cells of *S. enterica* serovar Typhimurium WT, Δ*sopD*, Δ*sseD*, and *E. coli* O157:H7 inoculated and dried on glass beads for 4 days at 38.5°C. Note the differences in cell morphology and matrix structure among the four strains. Arrows and letters indicate specific elements present in each strain:

matrix (m), fenestrations (f), cell concavity (c), and ropy filaments (r). The images are representative of the sample population. Magnification and scale bar are embedded in the images.

After 7 days of equilibration to a_w 0.11 (Fig 2.5), *Salmonella* WT cells were still characterized by the presence of an extracellular matrix, although the matrix was partially disrupted and detached. The cells maintained the rod morphology, but membrane corrugations and distortion, indicating loss of turgidity and cellular damage, were more evident than in the control. Both mutants, as well as *E. coli* O157:H7, lacked an extracellular matrix. Cells showed surface corrugation indicating membrane damage, and cell debris was present on the bead surface.

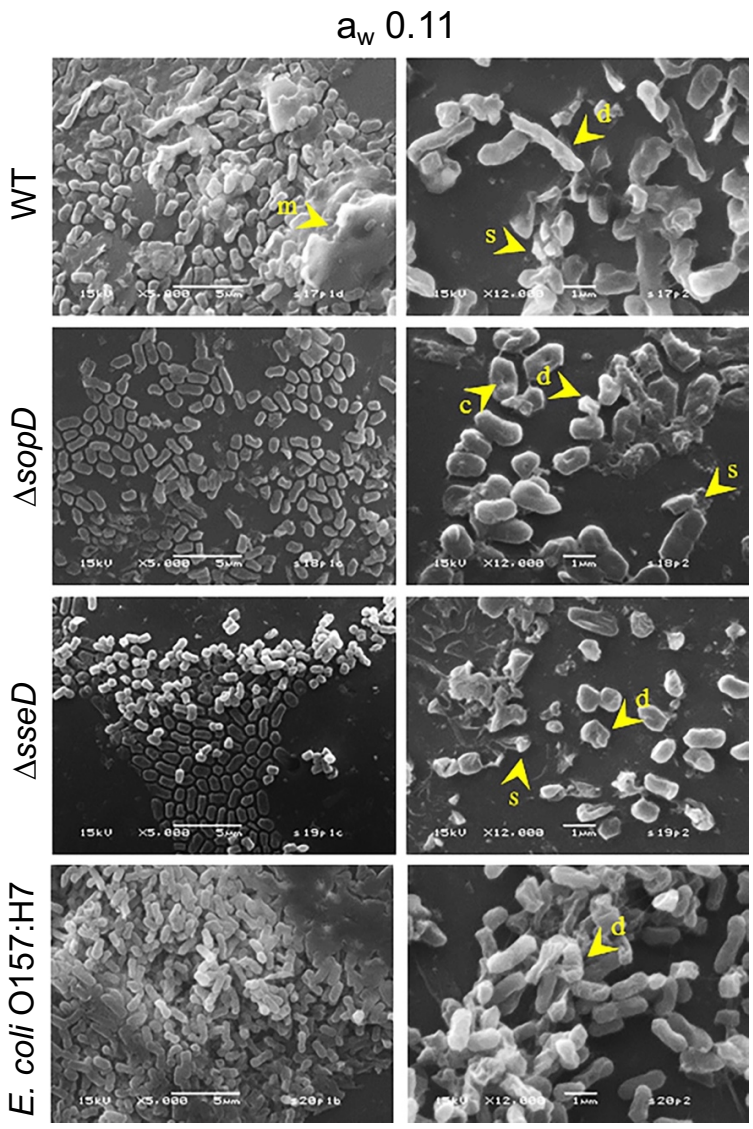


Fig 2.5. Scanning electron microscopy images of *S. enterica* serovar Typhimurium wild-type (WT), Δ*sopD* and Δ*sseD* strains, and *E. coli* O157:H7 after equilibration to a_w 0.11. Images of *S. enterica* serovar Typhimurium WT, Δ*sopD*, Δ*sseD*, and *E. coli* O157:H7 cells inoculated, dried and equilibrated for 7 days to a_w 0.11 on glass beads. Arrows and letters indicate specific elements present in each strain: matrix (m), cell concavity (c), damaged

cells (d), and debris (s). The images are representative of the sample population. Magnification and scale bar are embedded in the images.

As observed at a_w 0.11, after equilibration for 7 days to a_w 1.0 (Fig 2.6) the two mutants maintained the characteristic morphology and presented loss of turgidity and wrinkling of the membrane, suggesting cell damage. Cell debris was also observed on the beads for both mutants as well as for the WT and *E. coli* O157:H7. Differently than at a_w 0.11, the two mutants had an extracellular matrix similar to the one formed by the WT and *E. coli* O157:H7.

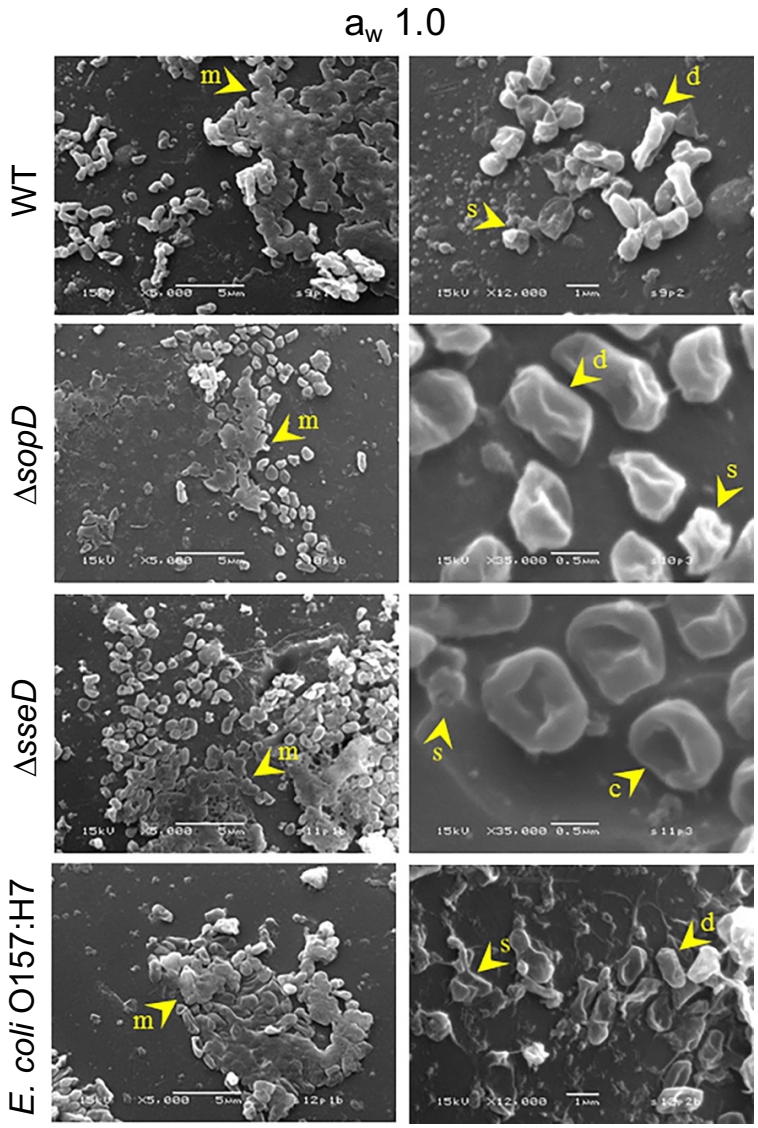


Fig 2.6. Scanning electron microscopy images of *S. enterica* serovar Typhimurium wild-type (WT), Δ*sopD* and Δ*sseD* strains, and *E. coli* O157:H7 after equilibration to a_w 1.0. Images of *S. enterica* serovar Typhimurium WT, Δ*sopD*, Δ*sseD*, and *E. coli* O157:H7 cells inoculated, dried and equilibrated for 7 days to a_w 1.0 on glass beads. Arrows and letters indicate specific elements present in each strain: matrix (m), cell concavity (c), damaged

cells (d), and debris (s). The images are representative of the sample population. Magnification and scale bar are embedded in the images.

2.5. Discussion

The response of *S. enterica* serovar Typhimurium to desiccation and exposure to low a_w is extremely important because it can trigger other, seemingly unrelated, stress tolerance responses (239, 287, 288, 313, 314), but its underlying molecular mechanisms are still largely unknown. Several groups have performed different global transcription analysis on desiccated *Salmonella*. Deng et *al*. studied the transcriptome of this microorganism by RNA sequencing in low a_w peanut oil (315) and there are a good number of reports on microarray-based transcriptomic analyses on *Salmonella* survival or adaptation to abiotic surfaces such as filter paper, stainless steel, and plastic (279, 280, 286). However, all of these studies have focused either on matrix-based low a_w or desiccation. To focus solely on the low a_w effect without the confounding factor of variations in the chemical composition of food matrices (e.g., batch to batch, sourcing of raw materials, and aging of the product), we decided to perform a global transcriptomic analysis on cells exposed to extreme low a_w using abiotic surfaces.

Our transcriptomic analysis showed that exposure to low a_w has a broad impact on the expression of many genes involved in anabolic and catabolic pathways. This was expected because the rate of enzymatic reactions slows

when occurring in low a_w, thus causing a decrease in the metabolic rate. This phenomenon has already been suggested in other studies (315). However, we also found that low a_w induced the expression of many genes involved in DNA replication and repair. DNA damage is known to be an effect of desiccation, in particular covalent modifications and double-stranded breaks (316). DNA repair genes were up-regulated under desiccation conditions in many different microorganisms, including *Deinococcus radiodurans* (317) and *Bradyrhizobium japonicum* (318). We can speculate that during the shift to low a_w, a portion of the replicating cells were unable to complete their replication due to either lack of energy and building blocks or water available for chemical reactions. This halt in the process would most likely be sensed as replication errors by the DNA replication checkpoints, thus inducing DNA repair mechanisms.

We also observed a stark increase in many tRNAs in cells exposed to low a_w. tRNAs are usually difficult to observe by RNA sequencing, mainly because of their strong secondary and tertiary structures and post-transcriptional modifications (319). Therefore, the observed increase in readable tRNA sequences for some tRNAs in desiccated cells might indicate that the post-transcriptional processing of those tRNAs is less efficient, possibly due to the same processes that cause DNA replication errors. However, an intriguing possibility is that the differential processing of tRNAs is a long-term adaptation strategy for the cell to coordinate amino acid transport and control translation. This would fit well with the high number of amino acid transporters and the

transcriptional/translational regulators we found induced at low a_w. Similar observations – up-regulation of amino acid transport and metabolism, and transcription and translation-associated genes – led Gruzdev et *al*. (286) to the conclusion that *de novo* protein synthesis is a requirement for the cell adapting to desiccation.

Osmolarity homeostasis is important in the desiccation process and, in our study, we observed the induction of *osmV* (2.91-fold), one of the genes involved in the transport of osmoprotectants during osmotic stress, in cells exposed to a_w 0.11 for four days. However, this was the only osmolarity-related gene induced in our treatment conditions. This is interesting, because one of the main long term responses the cell deploys to counteract osmotic stress during desiccation is the intracellular accumulation of osmoprotectants (320). Osmoprotectants can be accumulated intracellularly from the medium or by *de novo* synthesis (321-323). During desiccation, the ABC transporters ProU (ProVWX) and OsmU (OsmVWXY), in conjunction with the permease ProP, transport in the cytoplasm glycine/betaine and proline, the main osmoprotectants used by the cell (324-327). Li et *al*. and Finn et *al*. found the ProU system up-regulated in *S. enterica* serovar Typhimurium desiccated on filter paper and stainless steel, respectively (279, 280).

The difference we observed in the induction of the ProU system may be explained by differences in methodology, including desiccation conditions and length of exposure to low a_w. In particular, we washed and re-suspended the

cells in pure water prior to desiccation, whereas Li et *al*. (280) used cells in PBS and Finn et *al*. (279) used cells in LB. As suggested elsewhere (286), the use of isotonic solutions in this kind of experiments can be problematic, as it would induce osmotic response during desiccation through the increase of solute concentration caused by water evaporation rather than through a decrease in a_w. Moreover, those two studies focused on very short-term effects of desiccation (2 hours (280) and 4 hours (279)), whereas our analysis was performed after an extended period of time (4 days). Therefore, in our experiment, cells were already adapted to the initial osmotic shock and no longer needed many osmotic stress genes. These two non-exclusive explanations are corroborated by previous research that showed that 22 hour air-drying of water-resuspended *Salmonella* cells on plastic Petri dishes did not induce the Pro/Osm genes (286). It is important to mention that production of the ProU transport system is usually induced by the presence of environmental glycine or betaine, and in our case, as well as in Gruzdev's group, glycine was not present in the environment (328). A similar explanation can be applied to the absence of up-regulation of the trehalose biosynthesis genes (*otsA* and *otsB*), which is another important osmoprotectant synthesized by the cell (329-331).

Our class of orthology gene classification of the differentially expressed genes identified two genes encoding for virulence factors, *sseD* and *sopD*. While *sseD* and *sopD* have been characterized for their role in virulence and infection mechanisms, to our knowledge, they have never been reported to be involved in

desiccation adaptation and tolerance, and for this reason we targeted them for further analysis. SseD is a translocon component of the *Salmonella* Pathogenicity Island 2 (SPI-2) Type III Secretion System (T3SS) (332), and it is part of the *sseABCD* operon, encoding a chaperon protein and the other translocon components (333). We also observed the induction of *sseA*, encoding the chaperonin for *sseB* and *sseD* (306-308), and *sscA*, encoding the chaperonin of *sseC* (305), in cells exposed to a_w 0.11. This indicates that the entire SPI-2 T3SS translocon is likely involved during adaptation and survival to desiccation and low a_w. Interestingly, although SopD was initially identified as an effector translocated by the T3SS of SPI-1 (334), it is now suggested that the SPI-2 encoded injectosome can also be involved in its deployment (335). Even though SopD release is activated under SPI-1 inducing conditions (336), *sopD* expression remains elevated during later stages of infection and is involved in survival and replication inside the macrophage (335, 337).

To better understand the impact of these two genes on the ability of *S. enterica* serovar Typhimurium to survive desiccation, we compared the survival rates and morphological characteristics of the WT, Δ*sopD* and Δ*sseD* mutants, and a virulent strain of *E. coli* (O157:H7). We chose to include *E. coli* in our comparisons because of its genetic and physiological relatedness to *Salmonella* and lack of the same kind of virulence mechanisms encoded by the SPIs.

The mutants were first compared to the WT under optimal growth conditions (LBglc, 37°C, shaking), and no differences were detected in

generation time and in the ability to reach stationary phase. Additionally, the cell viability of the mutants in the initial cell-resuspension was checked, and the mutants, as well as *E. coli* O157:H7, had the same cell viability of *Salmonella* WT before being inoculated on glass beads (data not shown). On the contrary, interesting differences were detected when observing the different cell-resuspensions with SEM. Both mutants exhibited a shorter and coccoidal shape compared to the WT. Both Δ*sopD* and Δ*sseD* mutants had decreased tolerance to desiccation compared to the WT, clearly indicating that SopD and SseD play an important role during desiccation survival. To the best of our knowledge, this is the first time that different cell shape and size have been observed for mutants in these two genes.

In a previous study, field emission scanning microscopy (FESEM) was performed on a large number of SPI-2 effectors mutants and no changes in cell morphology were reported for Δ*sseD* in that case (338). The use of a different *S. enterica* serovar Typhimurium strain - ATCC14028 instead of NCTC 12023 - and different growth conditions might justify the different morphologies detected in our study. Furthermore, Chakravortty et *al*. used specific SPI-2 inducing conditions. As a result, the induction of other SPI-2 T3SS genes could have masked defects in cell morphology caused by the mutation in *sseD*.

A similar round shape was observed for *mreC* mutant in *Salmonella* (339) and *E. coli* (340). This gene belongs to the *mreBCD* operon, which is responsible for the cell shape and correct formation of the cytoskeletons (341-345). For *E.*

coli, deletion in *mreBCD* caused the formation of spheroid-like cells defective in adjusting the rate of phospholipid synthesis (340). Interestingly, we observed up-regulation of the entire *mreBCD* operon in WT cells of *S. enterica* serovar Typhimurium equilibrated to a_w 0.11, suggesting a role of the operon in the cell response to low a_w, probably through the maintenance of a correct membrane composition and cell-shape. Although we cannot directly prove a connection between *sopD*/*sseD* and the *mre* operon, the fact that the two mutants, defective in cell-shape, are less tolerant to desiccation, suggests that the desiccation response mechanisms may include the *sopD*/*sseD*-dependent induction of the *mre* operon.

The effects of the mutations persisted after the additional week at a_w 0.11. The cell viability of the two mutants decreased once more. This indicates that *sopD* and *sseD* are not only involved in *Salmonella* adaptation to desiccation but are also essential for long period survival. It is interesting to note that drying and exposure to a_w 0.11 had a dramatic effect also on *E. coli* O157:H7, which lacks SPI-1 and 2 genes.

An additional possibility is that the mutants are more prone than the WT to enter a viable but not culturable state (VBNC). The VBNC state is described as a dormant state in which cells are metabolically inactive and are not culturable using standard methods (346). This phenomenon has already been observed for *Salmonella* cells under different stress conditions, including drying and desiccation (6, 237, 347).

Structurally, after drying and prolonged exposure to a_w 0.11, *Salmonella* WT displayed a thick layer of solid extracellular matrix. In general, production of EPS has been associated to a higher desiccation tolerance in a variety of bacterial species, such as *E. coli*, *Acinetobacter calcoaceticus*, *Erwinia stewartii*, and *Rizhobium sullae* (348, 349). It is believed that EPS works as a water reservoir and is protective from desiccation (350). Moreover, genes encoding for fimbrial subunits were found induced in cells equilibrated to a_w 0.11 on filters - i.e. *sthA*, *sthB*, and *sthE*, part of the fimbrial operon *sthABCDE*, important for colonization in mice (351) as well as chickens (352). The matrix observed for *Salmonella* WT after the 7-day equilibration showed some signs of damage, possibly due to the desiccation of the hydrogel.

After drying, the extracellular solid matrices of both *E. coli* O157:H7 and Δ*sopD* presented signs of damage as well as cell detachment, while after exposure to a_w 0.11, neither Δ*sopD* nor *E. coli* O157:H7 had any kind of extra-cellular material production. Interestingly, Δ*sseD* did not produce any solid matrix after drying, although cells were encased in a network of ropy filaments, which appeared to connect all cells. Δ*sseD* did not present these filaments after exposure to a_w 0.11 and the cell viability decreased compared to after drying.

It is possible that the mutant cells can temporarily produce extracellular structures with the role of protecting the cell from desiccation, but this response might be ineffective for long periods of exposure to low a_w. Based on the different role and characteristics of SopD and SseD, we hypothesize that the decrease in

desiccation and low a_w tolerance that we observed in our mutants is mainly due to lack of secretion of the effector protein SopD. As previously mentioned, SopD is an effector secreted by both T3SSs, while SseD is part of the injectosome of the SPI-2 T3SS. SPI-1 and SPI-2 T3SS are expressed during the infection process at different stages (353). While SPI-1 expression is activated for the invasion of the host cell and the initial formation of the *Salmonella* containing vacuole (SCV), SPI-2 is necessary at a later stage, for *Salmonella* induced filaments (SIF) formation and bacterial replication inside the SCV.

In the case of the Δ*sopD* mutant, SopD cannot be produced nor secreted, and cell viability decreases dramatically from the first treatment (desiccation). Δ*sseD* is less susceptible than Δ*sopD* to the effect of initial desiccation, but after prolonged exposure to a_w 0.11, the effect of *sseD* depletion becomes as dramatic as *sopD*. These data suggest that during the initial desiccation, the SopD effector could still be secreted in the Δ*sseD* mutant, probably by SPI-1 T3SS, but during long-term low a_w exposure, the role of SPI-2 T3SS may become fundamental, and due to malfunctioning of the injectsome in Δ*sseD*, SopD may no longer be secreted. Additionally, the induction in the WT of *sseD*, as well as *sseA* and *sscA*, in cells equilibrated to a_w 0.11 strongly indicates that the correct assembly and functioning of the SPI-2 T3SS is required for survival at extreme low a_w conditions.

When cells were exposed to high a_w (a_w 1.0) after drying, cell viability for the WT, Δ*sopD*, and *E. coli* O157:H7 increased. This may be due to the ability of

the cells to utilize proteins and nutrients released by the dead cells still on the beads, similarly to what hypothesized by Gruzdev et *al*. (286). The presence of dead cells and cellular debris was observed by SEM after equilibration to a_w 1.0. Additionally, it has also been suggested that residues of extracellular polymeric substances (EPS) formed during desiccation can serve as a source of nutrients (233, 235). The Δ*sseD* mutant, instead, did not increase in cell viability after equilibration to a_w 1.0. The differences in recovery from desiccation between the two mutants could be linked to the different role of the two effectors. Possibly, the lack of a completely developed needle structure in Δ*sseD* results in defective secretion of several other effectors required for the recovery process. At a_w 1.0 no differences were detected in EPS production, confirming that the differences in the exopolymeric matrix formation between the WT and the mutants/*E. coli* O157:H7 are due to differences mainly in response to desiccation.

Very recently, the regulatory system of a 97 nt small antisense RNA, STnc3140, encoded on the negative strand of *sopD* and positioned in its coding region (from position +726 to +822), has been partially characterized in *Salmonella* (354-357). This sRNA was first named SLasRNA0334 and was identified in 2012 by Ramachandran et *al*. using a combination of differential RNA-seq and *in silico* analysis (356). A subsequent study published in 2013 by Kröger et *al*. renamed this antisense RNA STnc3140 and showed that this RNA interacted with the RNA chaperone Hfq specifically during the transition from late exponential to early stationary phase (357). In 2016, Colgan et *al*. thoroughly

characterized the regulatory networks of 280 sRNAs in 5 different conditions (mid exponential phase, intermediate exponential phase, early stationary phase, late stationary phase, and SPI-2 inducing) and found that STnc3140 is positively regulated by RpoS and Hfq, but is repressed by HilD and Fur (354). In particular, the authors of that study observed more than 3-fold decrease in STnc3140 expression in RpoS and Hfq mutants in late stationary phase and early stationary phase, respectively, and more than 3-fold increase in HilD and Fur mutants in early stationary phase. In a different study, Smirnov et *al*. observed a decreased expression of STnc3140 also in a ProQ mutant of *Salmonella* (355). In our mutant, this small RNA was removed along with the SopD coding sequence.

The absence of STnc3140 could be at least partially responsible for the low a_w-sensitive phenotype observed in the Δ*sopD*. However, this hypothesis is unlikely based on the fact that i) none of the regulators reported in the above mentioned studies are differentially expressed in our conditions; ii) the induction, under low a_w conditions, of *sopD* and few other genes related to secretion (e.g., *sseD*, *sseA*, and *sscA*) was described in the WT strain first and not limited to STnc3140; and iii) the phenotypes of Δ*sopD* and *ΔsseD*, although clearly distinct, they shared several similarities (e.g., coccoidal shape, sensitivity to desiccation and low a_w, impacted extracellular matrix production at low a_w). Regardless, as more information about small ncRNAs becomes available, it will be interesting to unravel the effects of the SopD effector from the ones of STnc3140, possibly by reintroducing into a Δ*sopD* background only STnc3140 and its regulatory unit

and complementing the Δ*sopD* mutation with codon bias-modified copy of the gene not encoding STnc3140.

3. Growth Conditions and Matrices Affect *Salmonella*'s Ability to Tolerate Desiccation and Thermal Treatment

3.1. Summary

Salmonella has the ability to survive desiccation and low water activity conditions (a_w < 0.6) becoming tolerant to heat. The main objective of the present study was to investigate the effect of matrices and growth conditions on desiccation survival and thermal tolerance of *S. enterica* serovar Typhimurium. *Salmonella* was grown in LBglc and M9 media, in the presence or absence of EDTA and dipyridyl. Cultures were inoculated on toasted oats cereals (TOC) or glass beads, dried, and equilibrated for a week at a_w 0.11 and 1.0, before being thermally treated at 75, 85, 90, and 95ºC. For all growth conditions and temperatures tested, cells exposed to a_w 0.11 had inactivation rates (δ-values) at least 10-fold longer than cells equilibrated at a_w 1.0. No statistically significant differences in the rate of inactivation were observed at either a_w 0.11 nor a_w 1.0 between the different growth conditions on TOC. For *Salmonella* grown in LBglc, the recovery after drying was significantly lower on glass beads than on TOC, as well as the thermal tolerance, suggesting that the food matrix is protective for desiccation and thermal treatment. On glass beads, cells grown in M9 were more susceptible to drying and low a_w than cells grown in LBglc, but had significantly greater δ-values at both a_w, indicating that nutrient availability during growth influenced *Salmonella*'s ability to survive desiccation and thermal tolerance.

Scanning electron microscopy showed that cells grown in LBglc and M9 displayed differences in the production of extracellular matrix, in particular during equilibration to a_w 0.11 and after thermal treatment at both a_w. Additionally, when *Salmonella* was grown on glass beads in LBglc as biofilm, the thermal tolerance was greater than cells dried on beads. Our observations suggest that growth in minimal media triggers cellular stress-responses, which might be implicated in the development of *Salmonella*'s thermal tolerance when desiccated.

3.2. Introduction

Salmonella is an enteric pathogen capable of surviving and colonizing many different environments, with an unique ability to cycle between abiotic exposure and periods of host internalization (358). Because of its resilience and widespread occurrence, *Salmonella* poses a major threat to food safety. As a result, strategies to avoid *Salmonella* cross-contamination, carryover of contamination after food processing, and elimination of *Salmonella* during processing, are very active areas of research in food safety (37, 38).

One of the emerging concerns of *Salmonella*'s viability in foods is the ability of this pathogen to survive for long periods in dry foods. Dry foods are characterized by a water activity (a_w) below 0.6, and have been historically considered microbiologically safe because bacteria are unable to grow at a_w lower than 0.88-0.91 (358, 359) and many bacteria tend to die off in very dry foods. This sense of security has been challenged by several outbreaks and

recalls over the past decade of many dry foods, including peanut butter, chocolate, flour, and red crushed pepper, due to *Salmonella* contamination (92, 188-190). *Salmonella* is not able to grow below a_w 0.94, but cells remain viable, albeit in a dormant state, for long periods of time (293, 360).

The processing of many dry food products often includes a step of thermal inactivation, but the presence of *Salmonella* in such products following thermal treatment has suggested that the conditions of the thermal treatment may be insufficient to kill this pathogen. When exposed to low moisture conditions, many serovars of *Salmonella* also displayed an elevated resistance to heat. This effect varies among strains and is dependent on many factors. For example, Ma *et al*. found that the thermal tolerance in peanut butter of three outbreak-associated strains of *S. enterica* serovar Tennessee was significantly greater than other strains of Enteritidis, Tyhphimurium, and Heidelberg, as well as clinical isolates of Tennessee (361).

Many diverse factors contribute to and influence the development of thermal tolerance (195). As mentioned, low a_w is a well-known and important physiochemical factor. Several studies have indicated that, in similar environmental conditions, a low matrix a_w corresponds to an increased thermal tolerance (215-217). For example, the inactivation time (D-value) of *S. enterica* serovar Enteritidis PT30 in almond kernels measured at 68ºC decreased from 6.97 min at a_w 0.72 to 0.96 min at a_w 0.89 and to 0.42 min at a_w 0.95 (218). Similarly, the D-value (measured at 90°C) of *S. enterica* serovar Enteritidis in

peanut butter was over 3-fold greater when the peanut butter was prepared at an a_w of 0.2 than 0.8 (215). A similar correlation was also observed between temperature and thermal tolerance; as the treatment temperature increased, the inactivation time decreased (218). For example, Villa-Rojas *et al*. observed a decrease of more than 9-fold in D-value at a_w 0.6 when the temperature was increased from 70ºC to 80ºC (D-values of 15.15 and 1.63 min, respectively) (218).

One of the challenges in determining the inactivation rates of *Salmonella* in food is due to the complexity of the matrices, that are very rarely homogenous. Moreover, additional factors like food composition, the content of fats, sugars, and salts (362, 363), or the presence of microenvironments can have a dramatic impact on the thermal tolerance and inactivation rates of *Salmonella* (216). *Salmonella* is more resistant to thermal treatment in fat rich foods, and fat is thought to be protective at high temperature because it may lower the a_w of the matrix due to an increased rate of molecular interactions between water and fat at high temperatures (226). Sucrose is another general component of food matrices that improves the thermal tolerance of *Salmonella* (221).

Less studied but still important factors that need to be considered are also the growth conditions of bacteria and their inoculation procedures in the food matrix. A recent study by Hildebrandt *et al*. reported that inoculation methods, regardless of the preforming laboratory, affected population stability and inactivation kinetics, thus reducing the reproducibility of the thermal inactivation

studies in wheat flour (364). It is important to remember that cells can also influence their surrounding environment through the production of biofilms. Cells that have formed biofilms have also been shown to have an increased thermal tolerance. Strains of *S. enterica* serovar Enteritidis that produce biofilm demonstrated a greater thermal tolerance than strains that did not produce biofilm when cells were inoculated on wheat-flour and equilibrated to a_w of 0.45 (228). Keller *et al*. also reported that thermal inactivation of both *S. enterica* serovar Oranienburg and Tennessee in low moisture peanut butter fit a linear model better when cells were derived from sessile cultures compared to planktonic cells (365).

Although the entirety of the desiccation-heat tolerance cross-protection mechanism is still far from being elucidated, it is now clear that a multitude of factors determines the fate of desiccated cells, and these include, but are not limited to, physical and chemical properties of the matrix, as well as cellular response mechanisms triggered by the changing environment. For instance, it is well known that genes belonging to osmotic stress, heat shock, and oxidative stress response pathways are activated during desiccation and thermal inactivation (247, 286). This study was undertaken to determine the effect of growth conditions, inoculation methods, and matrix differences on both *S. enterica* serovar Typhimurium's desiccation survival and thermal tolerance development. The ultimate objective was to better understand and further

advance the knowledge of some aspects that contribute to this pathogen's unique characteristic at low a_w.

3.3. Materials and methods

3.3.1. Bacterial strains and culture preparation

In this study, *Salmonella enterica* serovar Typhimurium strain ATCC 14028 (from now on, *S. enterica* serovar Typhimurium) was used as wild-type strain. The stock cultures and working cultures were maintained and prepared as previously described (Chapter 2, Materials and methods, section 2.3.1, page 51), but in addition to LBglc, M9 broth was also used (in 1 L, 20% M9 salts, 0.2% 1M $MgSO_4$, 2% 1M glucose, and 0.01% 1M $CaCl_2$).

3.3.2. Desiccation survival and thermal tolerance assessment on toasted oat cereal (TOC)

To determine the effect of chelating agents on *S. enterica* serovar Typhimurium thermal tolerance on toasted oat cereal (TOC), working cultures were inoculated into 3 different media: LBglc as control, LBglc containing 0.25 mM EDTA, and LBglc supplemented with 0.25 mM dipyridyl. To ensure adequate chelation of the ions, the chelating agents were added to LBglc media 48 h prior to inoculation. After overnight growth at 37°C with shaking at 250 rpm, cultures (100 mL) were centrifuged as previously described (Chapter 2, Materials and methods, section 2.3.6, page 56) in order to collect and wash cells. The washed pellet was re-suspended in 400 mL DSW and used to inoculate 30 g of TOC. The

inoculum/TOC mixture was left to soak for 2 min and was then filtered using sterile cheese cloth to remove excess liquid. For viable count enumeration, three pieces of TOC (each 0.07 g dry weight) were distributed into three 9-mL saline tubes (0.9% NaCl) and vortexed to homogenize samples. Serial dilutions and spread plating onto dTSA were used as previously described (Chapter 2, Materials and methods, section 2.3.6, page 56) in order to determine cell counts.

The inoculated TOC was dried in dryer at 38.5 ± 0.5°C for 4 days. Dried TOC was ground using a sterile blender jar, and ground TOC (0.05 g dry weight) was transferred into 0.2 mL polypropylene tubes. The tubes were placed inside desiccators at a_w 0.11 and 1.0 and allowed to equilibrate for 7 days, following the same procedure described for micro glass beads (Chapter 2, Materials and methods, section 2.3.6, page 56). Cell count was determined after drying and after equilibration, using the content of a single polypropylene tube (0.05 g dry weight).

For thermal treatments, tubes were removed from the desiccators and immediately sealed using their caps. The a_w of samples was determined before every experiment, and the samples were discarded if the a_w deviated more than ± 0.02 from the expected value. The sealed tubes were heat-treated using a block heater (VWR Digital Dry Block Heater, VWR International, Radnor, PA) at 3 different temperatures (85, 90, and 95°C). Cell viability was determined at regular time intervals removing tubes from the block, placing them in a water-ice bath for 1 min, and pouring the content of each plastic tube (0.05 g dry weight) into 9 mL

sterile saline test tubes. After mixing, 10-fold serial dilutions were prepared and spread plated onto dTSA. Inactivation times for both water activities were determined using the Excel add-in GInaFiT Version 1.6 (366) based on the Weibull model (212). The Weibull model yielded inactivation times as δ-values and the shapes of the inactivation curves were indicated by the parameter p (when p equals 1 the curve is linear, when p > 1 the curve is concave downward, when p < 1 the curve is concave upward). Additionally, for a_w 0.11, the data were fit using the Log-linear model (204) and D-values were calculated as $1/k_{max}$.

3.3.3. Cell viability experiments on micro glass beads

Cells cultures, beads inoculation and cell enumeration were performed as previously described (Chapter 2, Materials and methods, section 2.3.1 and 2.3.6, pages 51 and 56). Briefly, overnight cultures of *S. enterica* serovar Typhimurium were grown overnight at 37ºC shaking, cells were collected through centrifugations and washed with DSW, and the cell re-suspension was used to inoculate micro glass beads. Cell viability was determined through spread plating onto dTSA after 4 days of drying at 38.5 ± 0.5ºC, and after additional 7 days of equilibration at a_w 0.11 and 1.0.

3.3.4. Thermal tolerance assessment on glass beads

For thermal tolerance studies, the same protocol was used to inoculate, dry, and equilibrate the cells on glass-beads. D-values were determined at 75°C. Sealed plastic tubes were placed into a block heater (VWR Digital Dry Block Heater, VWR International, Radnor, PA) and, at regular time intervals, three

tubes were removed and placed into ice-water for one minute. The tube content was poured into larger tubes containing 9 mL sterile saline (0.9% NaCl), and serial dilutions were performed. Cell count was determined after plating dilutions on dTSA and incubating at 37°C for 24 h. Inactivation times were calculated as δ-values using the Weibull model Excel add-in GInaFiT Version 1.6 (212, 366) as described above for TOC.

3.3.5. Thermal tolerance assessment for biofilm on glass beads

Biofilm was grown by inoculating 20 µl of a *Salmonella* overnight culture in 20 mL of LBglc together with 20 g of glass beads in plastic tubes. The tubes were incubated at 37°C for 48 h while shaking at 50 rpm on a rocking shaker. After incubation, the beads were collected in filtration cups using a 5-µm membrane (GE water and Process Technologies, Trevose, PA). To remove free cells, or cells with a weak adhesion, 100 mL of DSW were used to rinse the beads. The beads were then put to dry for 4 days, and the same procedure used for planktonic cells was used for drying and equilibration to a_w 0.11 and 1.0. Cell count was determined with serial dilution and spread plating onto dTSA. Inactivation times were calculated as δ-values using the Weibull model Ecxcel add-in GInaFiT Version 1.6 (212, 366).

3.3.6. Scanning electron microscopy

The samples to be visualized by scanning electron microscopy were prepared as previously described (Chapter 2, Materials and methods, section 2.3.7, page 57). Briefly, immediately after collection, samples were fixed

overnight in a solution of 1% paraformaldehyde, 1% glutaraldehyde, and 0.05 M sodium cacodylate as previously described (302) and then dehydrated through an ethanol series and HMDS series. After the final step in 100% HDMS, samples were air-dried for 48 h at room temperature, and coated with 20 nm of gold-palladium after being placed on aluminum stubs with adhesive carbon tape. Scanning electron microscopy (SEM) imaging was performed with the JSM 6060LV scanning electron microscope (JEOL USA, Inc., Peabody, MA) using a 15 kV accelerating voltage. For this analysis, beads were collected after 4 days drying at 38.5 ± 0.5ºC, after 7 additional days of equilibration at a_w 0.11 and 1.0, and after thermal treatment at 75ºC for 51 min (a_w 0.11) and 29 s (a_w 1.0).

3.3.7. Statistical analyses

All statistical analyses were performed on results from at least three biological replicates for each condition for each experiment. Statistical significance was calculated as *p*-value using the two-tailed Student's t-test assuming equal variance among the experiments. Significance threshold was set at $p \leq 0.05$. The standard error of the mean (SE) was used to calculate variation among samples.

3.4. Results

3.4.1. Desiccation and thermal tolerance on toasted oat cereal

To test the effect of chelating agents on *Salmonella*'s ability to survive desiccation on a dry food matrix, TOC was inoculated with a suspension of *S.*

enterica serovar Typhimurium cells grown in the presence of dypirydil and EDTA. Cell viability was determined before and after drying for 4 days, and after 7 additional days of equilibration to a_w 1.0 and 0.11 (Fig 3.1).

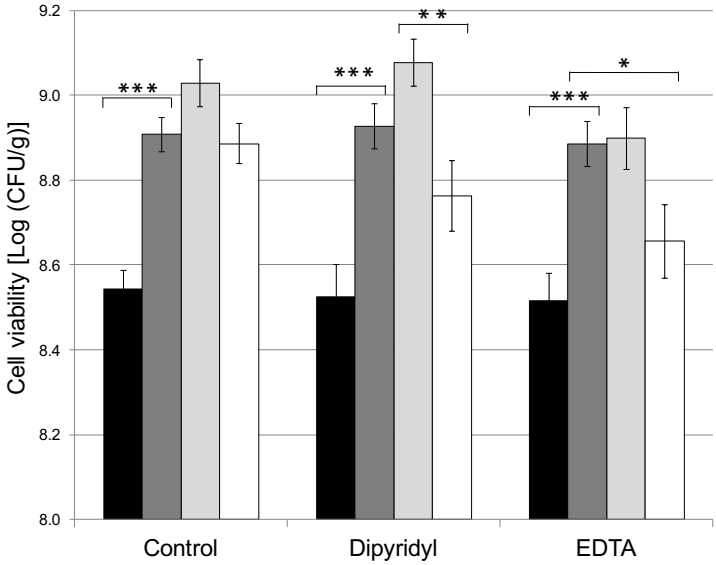


Fig 3.1. Viability of *S. enterica* serovar Typhimurium on toasted oat cereal (TOC) as affected by growth in the presence of chelators, before drying (black), after drying (dark gray), and after equilibration to a_w 1.0 (light gray) and 0.11 (white). *S. enterica* serovar Typhimurium was grown overnight at 37ºC in LBglc (control), or in LBglc containing 0.25 mM dipyridyl or 0.25 mM EDTA. TOC was dried for 4 days at 35˚C and equilibration lasted for 7 days. Bars indicate standard error of the mean (SE). Stars indicate *p*-values < 0.05 (*), < 0.01 (**) and < 0.001 (***) between the different treatments for each condition.

The initial inoculation level for the TOC was 8.5 Log (CFU/g) for all three conditions [LBglc (control), LBgcl+dipyridyl (didyridyl), and LBglc+EDTA (EDTA)]. For all three conditions, viable count increased to 8.9 Log (CFU/g) after drying and this increment, although small, was statistically significant (*p*-values less than 0.001). After the additional 7 days of equilibration to a_w 1.0, the count was slightly higher for control and dipyridyl samples, but these increases were not statistically significant (*p*-value > 0.05). No viability increase was observed in cells grown with EDTA. In contrast, after equilibration to a_w 0.11, the viability decreased for all conditions tested when compared to after drying, although the change was statistically significant only with EDTA (*p*-value = 0.026). The viability at a_w 0.11 was lower than at a_w 1.0 for all conditions, but statistical significance was observed only for dipyridyl (*p*-value = 0.006). Among the three conditions, no statistically significant differences were observed after any treatment, except for EDTA at a_w 0.11 compared to control LBglc (*p*-value = 0.034).

The effect of the chelating agents on *Salmonella*'s thermal tolerance on TOC was assessed (Table 3.1). The inactivation times were first determined using the Weibull model, which calculates the first Log reduction, and indicated as δ-values in minutes or seconds. In general, δ-values determined at a_w 0.11 were much greater than those determined at a_w 1.0, expressed in minutes and seconds, respectively. Moreover, δ-values decreased as the temperature increased in all conditions.

For control samples at a_w 0.11, the δ-value at 85ºC (240 min) was almost three times greater than the δ-value at 90ºC (81 min), with an even greater difference between 90 ºC and 95ºC (13 min), when the δ-value decreased more than 6-fold. Similar trends were also observed for dipyridyl and EDTA at a_w 0.11. Conversely, at a_w 1.0 the differences between the δ-values at different temperatures were slightly smaller than what was observed at a_w 0.11, for all the conditions. No statistically significant differences were observed at either a_w 0.11 nor a_w 1.0 among the different growth conditions. For all the conditions, p parameters, that indicate the shape of the curve, were calculated. All p parameters for the a_w 1.0 were above 1.0, indicating that the inactivation curves were all concave downward. On the contrary, p parameters for the inactivation curves at a_w 0.11 were all below 1, indicating that the curves were concave upward.

Table 3.1. Kinetics of inactivation of *Salmonella enterica* serovar Typhimurium on toasted oat cereal (TOC) after equilibration to a_w 0.11 and 1.0. Overnight cultures grown in LB glc (control) or in presence of dipyridyl and EDTA were inoculated on TOC. The Weibull model was used to calculate δ-values at both a_w. p indicates the coefficient of the curve. Different letters on the same row indicate *p*-values < 0.05 for the δ-values in different conditions.

a_w	Temp (ºC)	δ-values of cells grown at three conditions (units: min at 0.11 a_w; s at 1.0 a_w)														
		Control					Dipyridyl					EDTA				
		Mean	p	SE	R^2	R^2 adj.	Mean	p	SE	R^2	R^2 adj.	Mean	p	SE	R^2	R^2 adj.
0.11	85	**240^A**	0.80	17	0.93	0.90	**174^A**	0.76	43	0.98	0.97	**326^A**	0.93	84	0.93	0.90
	90	**81^A**	0.72	13	0.99	0.98	**77^A**	0.73	13	0.96	0.94	**88^A**	0.67	16	0.98	0.97
	95	**13^A**	0.58	3	0.99	0.99	**26^A**	0.72	4	0.97	0.96	**20^A**	0.62	5	0.98	0.98
1.0	85	**106^A**	3.93	8	0.99	0.98	**109^A**	2.94	10	0.96	0.94	**107^A**	3.86	3	0.96	0.95
	90	**72^A**	2.46	6	0.96	0.94	**85^A**	4.72	3	0.96	0.95	**75^A**	2.71	5	0.96	0.94
	95	**62^A**	2.14	4	0.97	0.95	**64^A**	2.69	5	0.97	0.96	**61^A**	3.09	8	0.98	0.97

SE: standard error of the mean
p: the coefficient of the curve

The inactivation times for a_w 0.11 were also calculated using the linear model, since linear inactivation is very often observed for low a_w (Table 3.2). In this case, the first Log reduction time (δ-value) equals the reduction time for the following Logs as well, and the parameter determined is called the D-value. The linear model confirmed that inactivation times decreased at higher temperatures.

For control samples, the D-value increased 3-fold when the temperature decreased from 95ºC to 90ºC, and more than 2-fold from 90ºC to 85ºC. A similar trend was also observed for dipyridyl. Differently, in EDTA a 3-fold increase was observed when the treatment temperature changed from 90ºC to 85ºC, but this difference was not statistically significant compared to the differences for the control and dipyridyl. As with the Weibull model, no statistically significant differences in thermal inactivation rates were observed due to the use of chelators.

Table 3.2. D-values *Salmonella enterica* serovar Typhimurium on toasted oat cereal (TOC) after equilibration to a_w 0.11. Overnight cultures grown in LBglc (control) or in presence of dipyridyl and EDTA were inoculated on TOC. Different letters on the same row indicate *p*-values < 0.05 for the D-values in different conditions. Stars (*) indicate significant difference between the D-value and the δ-value calculated for the same condition at the same temperature and a_w.

a_w	Temp (ºC)	D-values of cells grown at three conditions (min)								
		Control			Dipyridyl			EDTA		
		Mean	SE	R^2	Mean	SE	R^2	Mean	SE	R^2
0.11	85	**268^{A}**	9	0.92	**218^{A}**	24	0.95	**402^{A}**	63	0.91
	90	**123^{A}**	13	0.93	**113^{A}**	10	0.93	**130^{A}**	20	0.95
	95	**41^{A}**	10	0.93	**48^{A}***	6	0.94	**47^{A}***	6	0.94

SE: standard error of the mean

3.4.2. Effect of growth conditions on desiccation on glass beads

The effect of growth conditions on *Salmonella*'s ability to survive drying and desiccation on an abiotic matrix was studied by inoculating *S. enterica* serovar Typhimurium cells on micro glass beads, followed by drying and equilibration at a_w 0.11 and 1.0 (Fig 3.2).

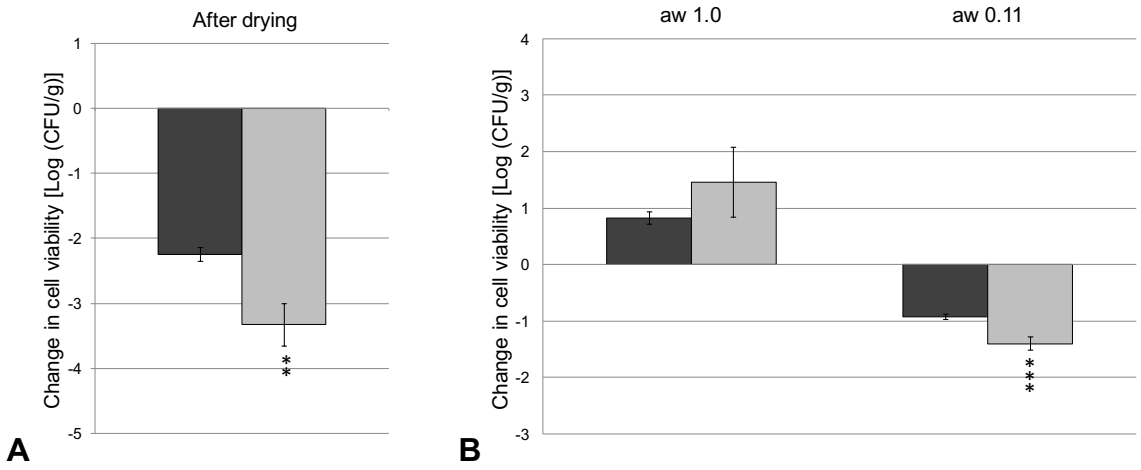


Fig 3.2. Changes in cell viability during drying and equilibration on glass beads. *S. enterica* serovar Typhimurium was grown overnight at 37ºC in LBglc (dark grey) and in minimal medium M9 (light grey). Change in cell viability were determined after 4 days drying at 38.5 ± 0.5°C (**A**), and after 7 days of equilibration at a_w 1.0 and 0.11 (**B**). Bars indicate standard error of the mean (SE). Stars indicate *p*-values < 0.01 (**) and < 0.005 (***) in comparison with LBglc after the same treatment.

While cell viability decreased for both conditions after drying (Fig 3.2 A), cells grown in M9 had a significantly lower survival compared to the LBglc-grown

cells [LB, -2.2 Log (CFU/g); M9, -3.3 Log (CFU/g)]. This difference was statistically significant (*p*-value = 0.006). After equilibration to a_w 1.0 (Fig 3.2 B), cell viability increased for both conditions [0.8 Log (CFU/g) for LBglc, 1.5 Log (CFU/g) for M9], and no significant difference was observed between M9 and LBglc. Differently from what was observed at a_w 1.0, after equilibration to a_w 0.11 (Fig 3.2 B), cell viability decreased in both conditions [-0.9 Log (CFU/g) and -1.4 Log (CFU/g) in LBglc and M9, respectively]. The decrease in cell viability for LBglc was significantly less than what was observed for M9 (*p*-value = 0.004).

The overall effect of the entire 11-day treatment, from inoculation to after equilibration, showed that there were no significant differences in the total changes in cell viability between M9 and LBglc (*p*-value = 0.14) at a_w 1.0. Conversely, at a_w 0.11 the total decrease for the LBglc was significantly less than what was observed for M9 (*p*-value = 0.0014, Fig 3.3).

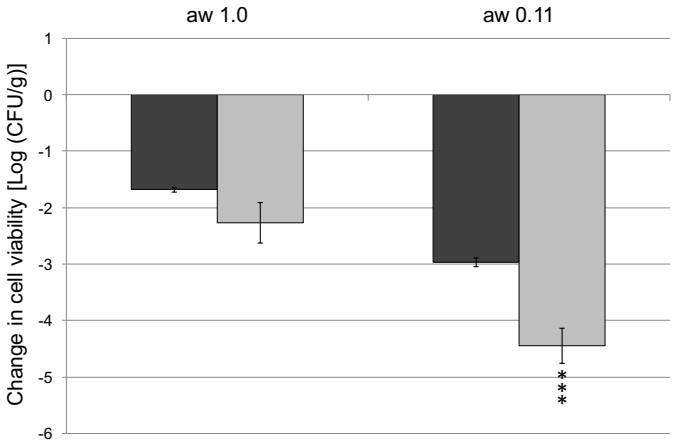


Fig 3.3. Total changes in cell viability during drying and equilibration on glass beads. Cells were subjected to 11 days of treatment (4 days drying and 7 days of equilibration to a_w 1.0 or 0.11) after growth in LBglc (dark grey) and M9 medium (light grey). Bars indicate standard error of the mean (SE). Stars indicate *p*-values < 0.005 (***) in comparison with LBglc after the same treatment.

3.4.3. Effect of growth conditions on thermal tolerance on glass beads

To remove the food matrix as a variable and reduce the complexity of the system, heat treatments were applied to cells inoculated on micro glass beads to understand the effect of pre-conditioning and growth conditions of *Salmonella*'s thermal tolerance. The cells were grown in three different conditions before inoculation: LBglc and M9 liquid cultures, and one sessile condition, in which *Salmonella*'s biofilm was grown in LBglc directly on the glass beads. Due to the higher thermal tolerance observed in literature for biofilm at low a_w, the

temperature of thermal challenge was increased from 75ºC to 95ºC, in order to have an observable reduction in Log (CFU/g).

The δ-values observed for a_w 0.11 were much greater than the values observed at a_w 1.0 (Table 3.3). For both a_w statistically significant differences were observed for δ-values between the LBglc and the minimal medium (M9) (*p*-values of 0.004 at a_w 0.11 and 0.03 at a_w 1.0). For the sessile cells (biofilm), the δ-value at 95ºC was significantly greater than the δ-values observed at a_w 0.11 for control at 75ºC (*p*-value = 0.022) (Table 3.3).

Table 3.3. Inactivation kinetics of *Salmonella enterica* serovar Typhimurium on micro glass beads after equilibration to a_w 0.11 and 1.0 as affected by growth media and biofilm. Overnight cultures grown in LBglc, in M9 medium, or as biofilms grown in LBglc were inoculated on micro glass beads. Thermal treatments were performed at 75ºC for cells, and at 95ºC for biofilms. The Weibull model was used to calculate δ-values. Different letters on the same row indicate *p*-values < 0.05 for the δ-values in different conditions.

a_w	Temp (°C)	δ -values of cells grown at three conditions (units: min at 0.11 a_w; s at 1.0 a_w)											
		LBglc				M9				Biofilm			
		Mean	SE	R^2	R^2 adj.	Mean	SE	R^2	R^2 adj.	Mean	SE	R^2	R^2 adj.
0.11	75	51[A]	7	0.97	0.95	255[B]	51	0.91	0.85	ND			
	95	ND				ND				128	37	0.86	0.79
1.0	75	29[A]	4	0.98	0.96	50[B]	7	0.95	0.92	ND			

SE: standard error of the mean

Scanning electron microscopy (SEM) was performed (Fig 3.4) to determine if changes in thermal tolerance between the pre-conditioning in LBglc and in M9 were due to changes in cell and extracellular structures. After drying (Fig 3.4, row 1), cells grown in either LBglc or M9 displayed the formation of an extracellular matrix. However, the two matrices looked different. While the cells grown in LBglc presented a smooth matrix that embedded cells in some parts, this was not homogeneously distributed on the surface of the bead. On the contrary, the matrix formed by cells grown in M9 completely enveloped most cells and covered the majority of the beads. The matrix looked rougher than the LBglc sample, and the cells were distributed on multiple layers, differently from the single layer observed in LBglc. In both samples, cells that were not enclosed in the extracellular matrix showed corrugated membranes and sign of cellular damage.

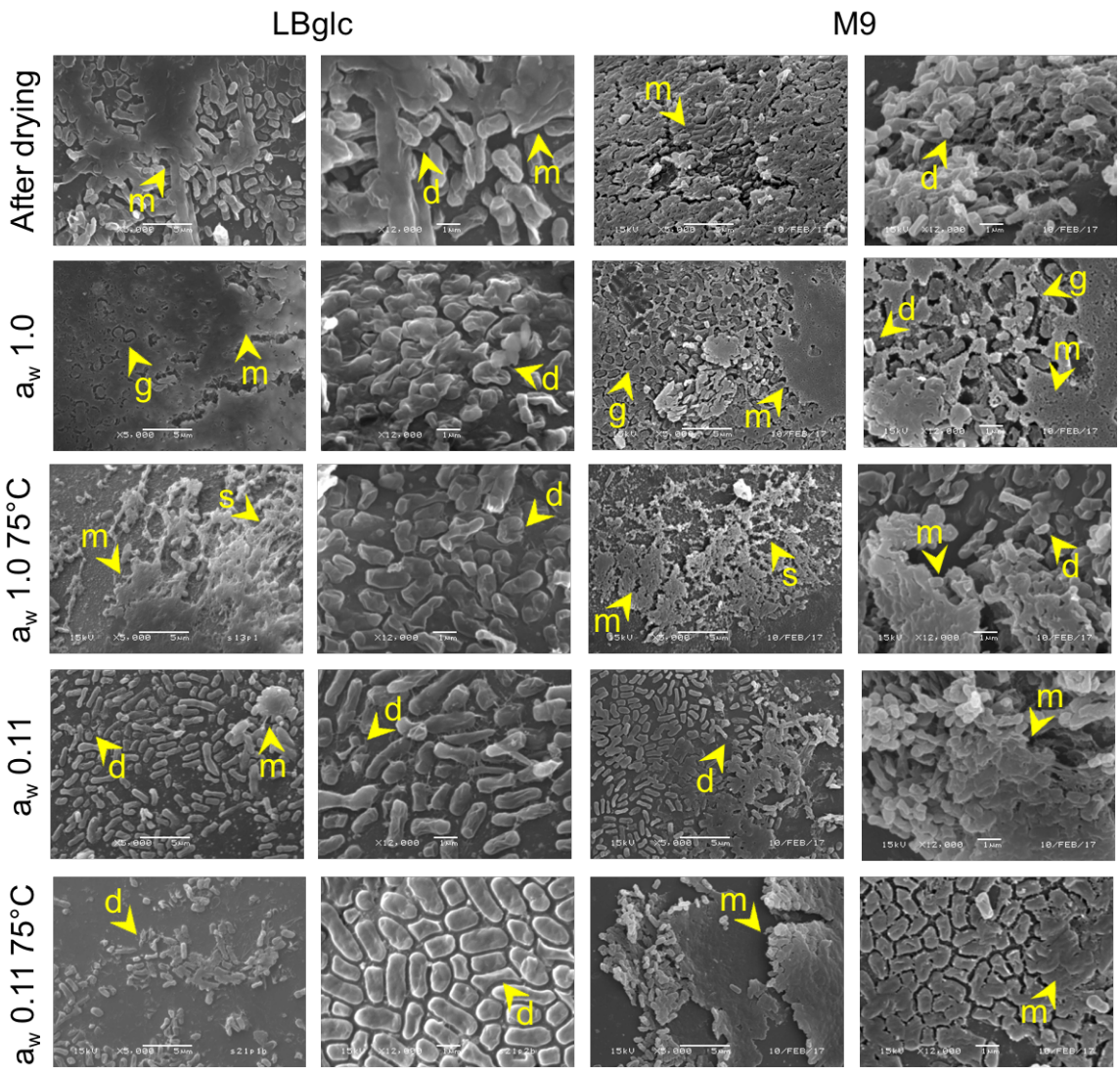


Fig 3.4. Scanning electron microscopy images of *S. enterica* serovar Typhimurium cells after drying, equilibration, and thermal treatment at 75ºC. Images of *S. enterica* serovar Typhimurium cells inoculated, dried for 4 days, equilibrated for 7 days to a_w 1.0 and 0.11, and thermally treated at 75ºC on glass beads. Arrows and letters indicate specific elements present in each sample after different treatments: matrix (m), gaps (g), cell damage and debris

(d), stringing of the matrix (s). The images are representative of the sample. Magnification and scale bar are embedded in the images.

After the additional 7 days of equilibration at a_w 1.0, both pre-conditioning treatments presented the same kind of extracellular structures observed after drying, but in both cases the extracellular matrices appeared partially flattened and damaged (Fig 3.4, row 2). Indeed, in the LBglc sample, the matrix lost thickness and three-dimensional structure, with gaps in the matrix where cells detached from the membrane. Similarly, in the M9 sample cells had detached from the top layer of the matrix, leaving gaps, and the matrix looked porous. In some areas, for both samples, the matrix remained intact and still fully enveloped the cells. Cell membrane corrugation and distortions were visible among cells that were exposed, indicating loss of turgidity and damage to the membrane. After thermal treatment, the matrix in both samples had lost solidity and it appeared stretched and shredded, which is an indication of damage to its structure (Fig 3.4, row 3). Cells were exposed, with distorted and wrinkled membranes, which indicates a loss of turgidity and cellular damage, and cellular debris was present. Differently from LBglc, in M9 a portion of the extracellular matrix was still three-dimensional, although in limited amounts.

After equilibration to a_w 0.11, clear differences were noticeable between samples grown in LBglc and samples grown in M9 (Fig 3.4, row 4). In particular, LBglc cells almost entirely lost their extracellular matrix. A single layer of exposed

cells covered the beads, and the cells appeared deflated and presented corrugation and wrinkling of the membrane, indicating loss of turgidity and cellular damage. After thermal treatment, the matrix was absent, and the density of the cells on the beads was clearly reduced (Fig 3.4, row 5). On the contrary, while the M9 sample presented exposed cells distributed in a single layer covering the beads similarly to what observed in the LBglc sample, the extracellular matrix was still present both before and after thermal treatment. The matrix still presented a multilayer and three-dimensional structure, with cells enveloped in it.

3.5. Discussion

In this study, we aimed to characterize the effect of growth conditions and different matrices on the ability of *Salmonella* to survive drying, desiccation at extremely low a_w, and to develop thermal tolerance. It has long been recognized that *Salmonella* can survive for long periods of time, up to several months, in dry conditions (191, 197, 218, 361, 367, 368). This ability is influenced by many factors, including the nature of the matrix, the availability of nutrients, and the storage temperature (195, 198-200, 216, 224, 362, 369). Once adapted to dryness, *Salmonella* becomes more tolerant to high temperatures (215-217), but this characteristic may be influenced by different environmental factors.

One of the main reasons for such an inconsistency in *Salmonella*'s behavior is because the cellular response to dryness and thermal tolerance involves many

different stress response systems, at both cellular, metabolic, and genetic levels (239, 370, 371). Although this complex response mechanism is far from being completely understood and described, it is widely acknowledged that the development of thermal tolerance is correlated with exposure to dry conditions and suboptimal a_w, and therefore the response system is better described as a cross-protection mechanism between stress responses. Some of the most important and best characterized responses involved in this cross-protection are osmotic, oxidative, and thermal shock responses (219, 239, 370, 371). While the role of osmotic and heat shock response system in the cross-protection mechanism is more direct and has been better elucidated, it is harder to fully determine the impact, and role, of oxidative stress.

Exposure to continuous osmotic and heat stresses in *E. coli* was found to induce expression of many genes belonging to oxidative stress (247). In particular, 26 genes of the SoxRS and OxyR oxidative-stress regulons were found up-regulated when *E. coli* was exposed to one of the two continuous stresses or the combination of the two. Among these were the entire *sufABCDSE* operon that encodes for an iron-sulfur cluster assembly pathway, and also *bfr* for bacterioferritin, the catalase *katE*, and the superoxide dismutase *sodC*. Since SoxRS is mainly activated in response to superoxide stress (superoxide radical anion O_2^-), while OxyR is mainly activated by peroxide stress response (H_2O_2) (372), these observations indicate that osmotic and heat stresses influenced the regulation of different oxidative stress response systems, and suggests that

oxidative response could be one of the missing links in the cross-protection mechanism.

Iron is directly involved in cellular oxidative stress. Indeed, due to its high redox activity, iron can take part in a variety of biological reactions that lead to the formation of radicals, which are responsible for cell damage (272). Some of these reactions involve physiological organic compounds, such as lipid oxidation, while others involve reactive oxygen species, which are a normal by-product of aerobic respiration (372). In particular, O_2^- generated during respiration can reduce Fe^{3+} to Fe^{2+} to form O_2, but it can also dismutate to H_2O_2, through either a spontaneous reaction in neutral pH aqueous environments, or through a superoxide dismutase (SOD) catalyzed reaction (372). H_2O_2 reacts with reduced metals, such as Fe^{2+}, in a reaction known as Fenton reaction, to generate hydroxyl radical ($OH^{\cdot}$) (372). Therefore, when the concentration of O_2^- increases, the concentration of $OH^{\cdot}$ increases as well, leading to damage of biomolecules and cell stress.

Regulation of free intracellular iron concentration is therefore fundamental to prevent and minimize oxidative stress. One of the strategies to control concentration of free iron is through the addition of chelating agents, i.e. multidentate ligands that can bind multiple copies of metal ions, such as Fe ions, therefore sequestering them from other biological reactions. To investigate the role of iron homeostasis in the cross-protection mechanism, we performed a series of experiments of desiccation, equilibration to low $a_{w,}$ and thermal

treatment using *Salmonella* cells grown in the presence of two different chelating agents: 1) dipyridyl, an intracellular chelating agent that forms complex with transition metals, such as Fe^{2+}; and 2) EDTA, an extracellular agent that sequesters metal ions such as Fe^{3+}.

The first matrix used was TOC, and both experiments of desiccation survival as well as thermal inactivation were performed. On the food matrix, we did not detect any significant different behavior of *Salmonella* cells, in terms of their ability to survive drying, as well as subsequent exposure to high and low $a_{w,}$ across the three growth conditions tested. Cells grown in control conditions (LBglc) and cells grown in the presence of chelating agents, both intracellular (dipyridyl) and extracellular (EDTA), were equally able to survive the first period of drying, therefore iron homeostasis is not important at this stage. Interestingly, we observed an increase in cell count that, albeit not dramatic, was statistically significant and indicated that the cells were able to use nutrients during the drying period from TOC as an energy source to grow.

Our findings were in some disagreement with previously reported results for *S. enterica*. Tamura *et al*. observed that in the presence of both lactoferrin and apolactoferrin, iron-binding enzymes, during drying at room temperature, the dry-resistance of six *Salmonella* serovars, including *S. enterica* serovar Typhimurium, was inhibited partially or entirely (373). In their protocol, cells were dried in a dryer at room temperature in the presence of lactoferrin or apolactoferrin in a solution of saline and 20% LB. It is probable that we did not

observe a marked effect because: 1) in our case the chelating agents were not added during the desiccation process; and 2) the food matrix was extremely rich in nutrients, and it not only allowed the cells access to energy sources, but it would have neutralized the effect of the chelating agents even if those were present during the desiccation.

Thermal treatment at three different temperatures (85, 90, and 95°C) was performed on *Salmonella* grown in control conditions and presence of dipyridyl and EDTA, and inoculated on TOC. δ-values, i.e. first Log reductions in cell count, were determined using the Weibull model (374) for a_w 0.11 and 1.0. As expected, based on previous reports, we confirmed that at all three conditions, the inactivation parameters (δ-values) decreased with increases in temperature, at both a_w (215, 217). At a_w 0.11, the δ-values were from 10- to 100-fold longer than at a_w 1.0.

The inactivation kinetics between the two a_w were very different. In fact, the analysis of the a_w 1.0 data showed that they best fit a downward concave curve, as determined by the Weibull parameter p, which indicates that the slope, and therefore the inactivation rate, increases with time. On the contrary, at a_w 0.11 the best-fit curves were just slightly concave upward, with p parameters below 1.0, indicating that the slope, as the inactivation rate, was decreasing with time. Moreover, since the p parameters were close to 1.0, we also analyzed the data using the Log-linear model. However, the R^2 of the linear models were still lower than the R^2 and R^2 adjusted obtained for the Weibull model, since the Weibull

model can adapt better to non-linear data. The linear model corroborated the Weibull's findings, and no significant differences were observed among the three growth conditions at a_w 0.11 at all the temperatures tested. The two models determined different inactivation parameters, but the differences were not significant at any temperature in any conditions, except for dipyridyl and EDTA at 95°C, suggesting that at higher temperatures, when the inactivation time decreases and the slope of the line increases, the Weibull model might be more accurate to describe the data.

To eliminate the effect of food matrix components on *Salmonella*'s desiccation and thermal tolerance assessment, we decided to change from a food matrix to an abiotic and inert material such as micro glass beads. Cells were inoculated on beads using a water re-suspension, and therefore negligible amounts of nutrients were available during drying and equilibration. The impact of nutrient availability was evident from the first drying step. Indeed, while cell count increased significantly during the 4 days drying when cells were inoculated on TOC, on glass beads we observed more than 2-Log reduction in cell viability, suggesting that, in absence of nutrients, cells died at a faster rate than in presence of nutrients.

Another factor to consider is the water retention capabilities of the matrix. Although both TOC and beads were completely dry after 4 days in the dryer (the a_w was 0.20 $\pm$ 0.5 in both cases), TOC was soaked in the water re-suspension solution, and water was evaporating slower in TOC than on glass beads, which

provided cells with more time for adaptation to osmotic stress and also with an opportunity to use nutrients from the food matrix for growth. Moreover, the presence of nutrients during the desiccation process is known to protect cells and increase the survival rate. A study from Hiramatsu *et al*. showed that after 24 h drying at room temperature, populations of 15 strains of *Salmonella* and STEC O157 and O26 were 100 times lower on paper disks compared to strains inoculated in dried foods containing sucrose and fat (197). Considering that our commercially available TOC contained more than 6 g of fat and 3 g of sugar for 100 g, in addition to proteins, vitamins, and minerals, it is not surprising to notice a similar protection observed by Hiramatsu *et al*. (197).

A similar effect was observed after equilibration to a_w 0.11. While the cell count remained constant in TOC, cell viability declined almost a Log (CFU/g) for cells equilibrated on glass beads, suggesting that the damages from prolonged exposure to dry conditions are less tolerated by cells exposed to prolonged starvation and in total absence of a protective matrix. On the contrary, after equilibration to a_w 1.0, cell availability increased, although just slightly, in both food and abiotic matrices, suggesting that when re-exposed to humidity, cells can use nutrients to grow. In the case of glass beads, where nutrients are not directly available from the matrix, we hypothesize, in accordance with Gruzdev et *al*. (286), that the energy sources could come from nutrients and proteins released by cells that died during desiccation and were thus still present on beads. This

hypothesis is also supported by the SEM observation of damaged cells and debris present at a_w 1.0 in control conditions (LBglc).

Thermal treatment of cells at both a_w 0.11 and 1.0 revealed that cells were inactivated on glass beads more rapidly compared to TOC. The temperature of treatment for TOC was 10°C higher than the treatment used for glass beads, but the δ -values in TOC were more than 4 times greater at 0.11 a_w, and more than 3-fold at 1.0 a_w. This confirmed that the food matrix, likely due to the presence of nutrients and the intrinsic structure of the matrix, is protective for *Salmonella* during thermal treatment, even in non-desiccating conditions.

Interestingly, on glass beads, growth conditions influenced the survival to desiccation and low a_w. Cells grown in minimal M9 medium had a significantly lower recovery than control cells grown in LBglc, both after drying and after equilibration to a_w 0.11. Since the only difference between the two conditions was the composition of the medium, we can speculate that the lack of nutrients during growth, such as proteins and amino acids, impacted the ability to promptly respond to desiccation and low a_w stress.

There are many factors involved in the bacterial response to growth in minimal media. While in LB and rich media cells mainly use amino acids as carbon source (375), in glucose minimal media, such as M9, all cellular building blocks are synthesized starting from sugar, inorganic nitrogen, and phosphate (376). Therefore, the anabolic demand is higher and the growth rate is reduced (377). One of the main consequences of growth in nutrient-limiting conditions is a

slow-down in growth rate due to starvation (378-380). In response to nutritional stress, protein and nucleic acid synthesis is downregulated. The global change in cell metabolism is initiated by accumulation of intracellular guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), a phenomenon known as stringent response (381, 382). The cytosolic levels of ppGpp and pppGpp are regulated by two enzymes, RelA, a synthase, and SpoT, a hydrolase (382, 383). Accumulation of uncharged tRNAs in response to amino acid starvation triggers the synthesis of ppGpp by RelA (384). ppGpp binds to RNA polymerases and prevents transcription of stable RNAs (rRNAs and tRNAs), which results in cessation of protein synthesis (385-387).

Additionally, ppGpp is also involved in positive regulation of some genes during stringent response. One of the main genes up-regulated by increase in the concentration of intracellular ppGpp is *rpoS* (388, 389), an alternative sigma-factor of the RNA polymerase (377, 380). ppGpp-free mutants (Δ*relA* Δ*spoT*) show a pleiotropic phenotype similar to *rpoS* mutant, and overproduction of ppGpp results in increased RpoS levels (388). Transcriptional analyses of bacteria grown in rich and minimal media have revealed that a number of genes are differently regulated between the two conditions (376), and RpoS has been indicated as one of the main factors contributing to this diversity (380). While RpoS is kept silent during growth in rich medium (e.g. LBGglc) by transcriptional and post-transcriptional regulation, during stress response, such as during growth in minimal media, instead, RpoS is highly expressed.

One of the main activation points of RpoS is the onset of stationary phase, which happens in both rich and minimal medium, when RpoS controls about 10% of the genome in *E. coli* (390), but there are many sets of genes that are regulated by RpoS in specific conditions (391, 392). A study from Dong *et al.* showed that in minimal medium during stationary phase, the expression of about 225 genes was RpoS-dependent, and the genes belonged to pathways involved in ribosome formation and maturation (*rimM* and *rmf*), protein folding and degradation, including genes for chaperone proteins (e.g. *dnaKJ*, *cbpA*, *groEL*), genes for modification of rRNA and tRNA (trmD, fmt, miaA), and genes for tRNAs (*argZ*, *lysW*, *glyT*, *serV*, and *alaT*).

One possibility is that the difference in desiccation and low a_w survival that was observed between M9- and LBglc-grown cells could be due to activation of RpoS. Since RpoS is responsible for the activation of many genes in stationary phase in minimal medium, we can assume that in cells grown in M9 many of the stress-response systems are already active, or at least some of the components of those pathways have already been synthesized. If that was true for every cell in the culture, we would observe an opposite effect of drying, as well as low a_w, i.e. a higher recovery of the M9 sample compared to the LBglc. A possible explanation is that not all the cells in M9 were adapted to stationary phase. Indeed, when the cells were inoculated on the beads, the cultures were in stationary phase, and therefore the cell cycles were not synchronized. This could translate in the presence of cells that are already adapted to stationary phase,

and cells that are still adapting and have not yet triggered a complete stress response. These cells would be more susceptible to stress in M9 compared to LBglc, especially due to the higher anabolic demand in M9 compared to LBglc, as well as the lower rate of protein synthesis and ribosome formation. If this was true, this portion of the M9-grown cells would be more susceptible to desiccation and die faster compared to the same portion of cells in LBglc, which could explain the lower recovery after drying and equilibration to low a_w observed for the M9 samples.

This hypothesis is supported also by SEM observations of cells equilibrated at a_w 0.11. Although M9 samples showed a noticeable thicker and more widely distributed matrix compared to the LBglc samples, cell damage and debris were present in both samples. Moreover, the matrix did not cover the entirety of the cells in either sample, both after drying and after equilibration to a_w 0.11. This could be a sign that a portion of cells, those not imbedded in the matrix, were non-adapted, i.e. they were unable to start producing EPS and form an extracellular matrix. This portion of cells would be more susceptible to desiccation and low a_w, in both samples, because they lacked the protection of the extracellular substance. In the case of M9 grown cells, however, these non-adapted cells could die faster than equivalent portion in the LBglc sample, as mentioned above, due to their weaker metabolic and nutrient state caused by growth in nutrient-poor conditions.

No differences in recovery were observed at a_w 1.0 between the two media, when cell viability increased. When observed with SEM, both samples displayed the presence of damaged cells and debris, as well as the presence of EPS. As previously mentioned, higher cell count could imply cell growth, thanks the use of nutrients derived from dead cells or from the degradation of the EPS, as previously suggested (233, 235).

Cells grown in M9 were more thermally tolerant at both a_w, with δ-values significantly greater than those measured for cells grown in LBglc. As previously mentioned, the presence of nutrients during desiccation and exposure to low a_w is a protective factor for *Salmonella*, but links between nutrient availability during growth and development of thermal tolerance have yet to be elucidated. To our knowledge, this is the first report that observed the effect of culturing in minimal media on *Salmonella*'s ability to survive desiccation and develop thermal tolerance. The longer inactivation times observed for M9-grown cells at both a_w suggested that the increased tolerance to heat treatment is correlated to a pre-adaptation to stress developed through culturing in M9 and is independent from the a_w of the matrix during the thermal challenge. This may not be surprising, since it is well known that pre-exposure to mild stress can be protective for heat treatment. For example, acid-adaptation and NaCl exposure have been shown to confer a higher thermal tolerance to *Salmonella* cells (393, 394), and carbon starvation has been linked with higher thermal tolerance in *E. coli* (395).

At a_w 1.0, both M9- and LBglc-grown *Salmonella* presented an extracellular matrix and EPS production before thermal treatment. After thermal treatment (75°C), the micrographs taken from M9 sample showed signs of cellular damage and stringing, as did the LBglc sample. However, the M9 sample still had areas of thick and undamaged matrix. This could indicate that the EPS formed by M9 cells at a_w 1.0 was less susceptible to thermal damage than the EPS formed by cells grown in LBglc, which could explain the increased in thermal tolerance for the M9 sample. Similarly, at a_w 0.11 the EPS matrix formed by the M9 sample was still intact after thermal treatment, while the LBglc sample, which had a lower production of EPS before thermal treatment, appeared to have lost any kind of matrix production following thermal challenge, and subsequently presented a lower cell viability.

Interestingly, cells grown to form biofilm in rich medium (LBglc) had longer inactivation times at a_w 0.11 than free cells at a 20˚C lower temperature. EPS formation and biofilm are known to confer protection to cells during various stresses (228, 294, 396). Production of fimbriae and curli are protective for desiccation and survival in dry conditions for long periods in *Salmonella* (294, 396). Recently, Villa-Rojas *et al*. demonstrated that more biofilm production in *Salmonella* on wheat flour confers higher thermal resistance, although their study did not find any correlation between biofilm-forming abilities and survival in desiccation (228).

It is well known that biofilm formation and cellular adherence increase during nutrients limitation, such as those encountered in M9 (397-400). Oh *et al*. demonstrated that on glass surfaces, *E. coli* O157:H7 forms more biofilm, and at a faster rate, when grown in minimal media M9 compared to LB (398). Similarly, stimulation of biofilm formation in several *E. coli* strains was found to be stronger in minimal media than in LB (397). The general stress-response sigma factor σ^{38} (*rpoS*) is involved in biofilm formation and maturation. For example, *rpoS* mutants in *E. coli* could not establish a mature biofilm on glass beads (401), and were found incapable of establish sessile communities in flow chambers (402). Interestingly, among the genes up-regulated by σ^{38} in stationary phase, Dong *et al*. also found another sigma factor, σ^{24} (*rpoE*), to be up-regulated [49]. This alternative sigma factor regulates expression of genes involved in the response to high temperature [58], extracytoplasmic stress [59], and starvation [60]. Moreover, a recent study observed a significant up-regulation of *rpoS* in *Salmonella* cells adapted to desiccation upon 3, 12, and 24 h (403). Furthermore, in the same study, *rpoS* mutants, both desiccated and not, showed a decreased tolerance to thermal treatment, compared to desiccated and non-desiccated wild-type, indicating that mutations in *rpoS* could lead to the loss of thermal tolerance in *Salmonella* (403). These observations lead us to speculate that the higher thermal tolerance and greater EPS production observed in M9-grown cells is due to a change in the cell physiology caused by growth in minimal medium and

mediated by σ^{38}, and which results in activation of multiple stress response pathways.

4. iTRAQ-based Global Proteomic Analysis of *Salmonella enterica* serovar Typhimurium in Response to Desiccation, Low a_w, and Thermal Treatment

4.1. Summary

The ability to survive desiccation and develop thermal tolerance is a well-known trait of the genus *Salmonella*. Several studies have focused on the low water activity (a_w) transcriptome of this pathogen when inoculated in different food matrices or on abiotic surfaces, but there is a lack of proteomic analyses in the literature. The objective of this study was to evaluate the changes in the global proteome of *Salmonella* in response to desiccation and thermal treatment, using the iTRAQ multiplex technique. *Salmonella enterica* servorar Typhimurium was dried, equilibrated at high a_w (1.0) and low a_w (0.11), and thermally treated at 75°C. The proteomes were characterized for cells after inoculation on glass beads (Day 0), after drying, after equilibration to high and low a_w, and after thermal treatment at both a_w. The Scaffold Q+ comparisons of the different protein profiles for each treatment identified 734 proteins differentially expressed among samples. After PCA analyses and hierarchical clustering, a group of 175 proteins was identified as the main source of the variation observed between the proteomes of the different treatments. PCA and hierarchical clustering determined that the samples were clustered into two major groups, based on their proteomic expression profiles, “dry” samples and “wet” samples. In general,

both groups of samples showed changes in the levels of proteins involved in DNA repair, replication, transcription, and translation, confirming that cells at both conditions need to strictly control the rate of replication and protein synthesis. Similarly, shifts in the presence of metabolic enzymes were observed for both “dry” and “wet” samples, indicating a switch in the energetic fluxes in response to different stresses. Proteins with higher expression levels in wet samples included motility proteins, specifically flagellar proteins (FlgE, FlgF, FlgG, FlgH), membrane proteins and export systems (SecF, SecD, the Bam complex). Interestingly, stress response proteins were more abundant in “wet” than in “dry” samples thus suggesting that rehydration can trigger stress responses in “wet” cells. “Dry” samples had higher levels of ribosomal proteins, from both 50S and 30S subunits, indicating that ribosomal proteins might be important for extra-ribosomal regulation of cellular response even when the synthesis of proteins is slowed down. No significant differences in protein expression were observed between the thermally treated samples and not, at both a_w. In conclusion, our study indicated that pre-adaptation to dry condition was linked to increased thermal tolerance, while reversion from a dry state into a wet state implied a significant change in protein expression that is linked with the loss of thermal tolerance.

4.2. Introduction

Salmonella enterica is the main cause of outbreaks and hospitalizations for bacterial foodborne diseases (75). This bacterium can survive, and even

thrive, in diverse and stressful conditions, ranging from acidic to basic pH, low and high temperatures, and low moisture conditions, characterized by water activities (a_w) lower than 0.6 (4, 6, 358). As a result, this bacterium is widely distributed in the environment and, because of its ability to tolerate harsh treatments and sublethal environmental stresses, it can inhabit food production facilities, which poses a relatively high risk for cross contamination of food products (38).

The ostensible increase in *Salmonella* outbreaks number linked to consumption of dry foods has raised scientific awareness on *Salmonella*'s ability to survive in low a_w conditions for extended periods of time. Various studies have been published on this topic and the picture they report is concerning from the public health perspective. For example, *Salmonella* has been shown to survive for an extended time in many different dry food matrices such as peanut butter (191), skim milk powder (200), whole black peppercorns and cumin seeds (238), and flour (404). In addition to food matrices, *Salmonella* has also been shown to survive for more than 100 weeks on plastic abiotic surfaces at 5°C (237) and on stainless steel discs for at least 30 days at 25°C (196). These findings support the idea that *Salmonella* can persist in many different dry foods and environments, potentially leading to contamination events and subsequent outbreaks.

During desiccation, water evaporates leading to a weakening of hydrophobic interactions. This phenomenon can cause the destabilization of the

cellular membranes, denaturation of the proteins, and eventually cause plasmolysis. As a result, the cell activates a complex system of cellular responses aimed to minimize this damage (240). Intracellular accumulation of osmoprotectants, low molecular weight solutes such as betaine and glycine, is the first line defense deployed to retain intracellular water (329). Amongst these solutes, trehalose, a disaccharide, seems to play an important role in the desiccation response. This carbohydrate has been found to decrease intracellular fluidity through a process called vitrification. Removal of water from a system results in the disaccharides entering a glass-state, hence the name of the phenomenon. This phase is associated with a decrease in the diffusion and accumulation rates of reactive oxygen species (ROS), thus slowing the deterioration of cellular components (405, 406). In a transcriptional study by microarray on desiccated *Salmonella*, Li *et al*. observed up-regulation of *otsB*, a gene involved in trehalose biosynthesis (280). Supporting this observation, they also measured a significant increase in trehalose concentration after cells had been equilibrated to a_w 0.11 for 5 days (280). More recently, a study investigating the transcriptome of desiccated cells found that also the ProU and OsmU systems, involved in cellular osmoregulation, were up-regulated in low a_w conditions and in dried cells (279).

Salmonella exposure to desiccating conditions triggers cross-protection against other environmental stressors. This is a major public health concern, but, more specifically, it is a threat for the food industry because increased thermal

tolerance is one such cross-protection allowing the bacterium to survive heat treatment for extended periods of time (3, 407). Unfortunately, increasing the intensity and/or time length of the heat treatment is not the best solution because of its adverse impact on the organoleptic and nutritional characteristics of the food product. The mechanisms triggering this cross-protection in *Salmonella* remain largely unknown. It has been suggested that this trait could arise from the absence of water, and, therefore, a reduction in intracellular molecular mobility. As a consequence, this would stabilize the structure of the ribosomal subunits (232). Destabilization of the ribosome subunits is hypothesized to be one of the main causes for bacterial inactivation during exposure to elevated temperatures (229, 231). There are other factors that are possible culprits, such as the extracellular matrix produced by the cells during biofilm formation (228) and the components of the food matrix in which the cells are exposed to the treatment (408). In fact, biofilm-forming strains of *Salmonella* Enteritidis inoculated in wheat flour were shown to have a greater thermal tolerance than strains that did not produce biofilm (228). In peanut butter, higher fat content and lower carbohydrate content corresponded to an increased heat resistance of *Salmonella* (408).

There is broad consensus in the literature that osmotic, thermal, and oxidative stress response systems might overlap and determine *Salmonella*'s ability to adapt to dry conditions and develop thermal tolerance (239). A recent study in *E. coli* demonstrated that genes part of the oxidative stress regulons *oxyR* and *soxRS* were induced during exposure to osmotic stress and/or high

temperature (247). Moreover, adaptation to acidic conditions also induced higher thermal tolerance in *Salmonella* cells, by modifications of the membrane composition (264). Thermal shock response in *Salmonella* is mainly regulated by two sigma factors (248-250), σ^H (*rpoH*), acting at the cytoplasmic level and inducing the synthesis of protein chaperons, and σ^E (*rpoE*), which regulates the response on a extra-cytoplasmic level, protecting the membrane from damage by detecting and repairing misfolded OMPs in the periplasm (248, 255-257, 409). Interestingly, it has been shown that the interplay between σ^E and σ^H requires also the general stress response σ^S (*rpoS*) (268). Clearly, these observations show that cellular responses to different stresses, like those encountered during desiccation and thermal treatment, require the activation of multiple cellular regulators.

The span of the components of these responses is yet to be fully characterized. Although numerous transcriptional studies conducted in the past decade on desiccated and thermally treated *Salmonella* have elucidated some aspects of this phenomenon, there is a lack of information at the proteomic level. This would provide an important tassel to our understanding of the final response activated by the cell. Among the different proteomic techniques, the iTRAQ is better suited to analyze the proteome of different samples. iTRAQ is based on the use of isobaric tags to uniquely identify up to 8 different samples (410), thus allowing the analysis of multiple samples at the same time (multiplex). In this work, we used this methodology to characterize the proteomic profile of

Salmonella's cells as affected by desiccation, exposure to very low a_w, and thermal treatment.

4.3. Materials and methods

4.3.1. Bacterial strains and culture preparation

The strain used in this study was *Salmonella enterica* serovar Typhimurium strain ATCC 14028 (from now on *S. enterica* serovar Typhimurium). The stock culture and the working cultures were prepared in 0.01 M glucose-supplemented LB broth (LBglc) as previously described (Chapter 2, Materials and methods, section 2.3.1, page 51).

4.3.2. Inoculations, desiccation, and thermal treatment on micro glass beads

The procedure followed for bead inoculation, drying, and equilibration to a_w 0.11 and 1.0 was the same as previously described (Chapter 2, Materials and methods, section 2.3.6, page 56), while thermal treatment was performed following the protocol previously illustrated (Chapter 3, Materials and methods, section 3.3.4, page 93). In summary, overnight bacterial cultures were collected through centrifugation, washed with DSW, and inoculated on micro glass beads (Day 0). The beads were dried for 4 days at 38.5 ± 0.5ºC and then equilibrated to a_w 0.11 and 1.0 for 7 days at 25ºC. Thermal treatment was performed at 75ºC for 29 s (a_w 1.0) and for 51 min (a_w 0.11), times corresponding to the previously calculated ∂-values. After every treatment, beads were snap frozen using liquid

nitrogen, and then the samples were immediately transferred into a -80ºC freezer, where they were stored less than 4 weeks before being processed for extraction and LC-MS at the University of Minnesota Center for Spectrometry and Proteomics (College of Biological Sciences, St. Paul, MN).

4.3.3. Protein preparation, proteolytic digestion, and iTRAQ labeling (performed by the UMN Center for Spectrometry and Proteomics)

Aliquots of 400 µl of extraction buffer [7 M urea, 2 M thiourea, 0.4 M triethylammonium bicarbonate (TEAB) pH 8.5, 20% acetonitrile, and 4 mM Tris [(2-carboxyethyl) phosphine hydrochloride (TCEP)] were added to frozen glass bead samples. All samples were vortexed for 15 s then incubated at 37°C for 1 h. Methyl methanethiosulfonate (MMTS) was added to each sample to a final concentration of 8 mM. The samples were vortexed briefly and incubated at room temperature for 30 min. After incubation, the samples were centrifuged at 12,000 x g for 10 min and the supernatant of each sample was transferred to a new 1.5 mL snap-cap microfuge tube. A Bradford assay was done to determine concentration of each sample. Proteolytic digestion, iTRAQ 8plex labeling and HPLC fractionation were carried out as previously described (411). Two sets of iTRAQ 8plex (each for a biological replicate) were made with replicate internal controls (pooled samples) in each iTRAQ set. Every sample was labelled with isobaric tags as indicated in Table 4.1. The tags bind to the N-terminal amine and the side chain of lysine residues, which ensure the labelling of every peptide in

the digested mixture (410, 412). After labeling, the samples within each iTRAQ set were multiplexed together and processed as described previously in Anderson *et al* (411).

Table 4.1. Labeling scheme for the samples of *Salmonella* cells subjected to drying, two water activity levels and thermal treatment in iTRAQ 1 and 2.

iTRAQ 8plex 1								
iTRAQ label (*m*/*z*)	113	114	115	116	117	118	119	121
Sample	Pooled control	a_w 0.11 thermal	a_w 1.0 thermal	Pooled control	a_w 0.11	a_w 1.0	Day 0	After drying
iTRAQ 8plex 2								
iTRAQ label (*m*/*z*)	113	114	115	116	117	118	119	121
Sample	a_w 0.11	Pooled control	a_w 1.0	a_w 1.0 thermal	After drying	Pooled control	Day 0	a_w 0.11 thermal

4.3.4. Liquid chromatography-mass spectrometry (LC-MS) (performed by the UMN Center for Spectrometry and Proteomics)

We analyzed fractions obtained from the first dimension of LC separation by online capillary LC-nanoelectrospray-MS on an Orbitrap Velos MS system (Thermo Fisher Scientific, San Jose, CA) as previously described (413) with the following exceptions: HCD activation time was 20 ms; lock mass was not employed; dynamic exclusion settings were: repeat count = 1, exclusion list size

= 500, exclusion duration = 30 s, exclusion mass width (high and low) was 15 ppm and early expiration was disabled.

4.3.5. Database searching (performed by the UMN Center for Spectrometry and Proteomics)

Tandem mass spectra were extracted by Proteome Discoverer Software (v. 2.1.0.81, Thermo Fisher Scientific, San Jose, CA, USA). Charge state deconvolution and deisotoping were not performed. All MS/MS samples were analyzed using Sequest v. 2.1.0.81 (Thermo Fisher Scientific, San Jose, CA, USA). Sequest was set up to search *S. enterica* serovar Typhimurium (strain LT2 and ATCC 14028) protein FASTA sequences downloaded from UniProt on June 21, 2016 after concatenation with the common lab contaminants database from http://www.thegpm.org/crap/ for a total of 6068 protein sequences. Sequest was searched with a fragment ion mass tolerance of 0.100 Da and a parent ion tolerance of 50 ppm. The methylthio group of cysteines was specified in Sequest as a fixed modification. Pyro-glutamic acid, deamidation of asparagine, oxidation of methionine, dioxidation of methionine, and iTRAQ 8-plex of lysine and peptide N-terminus were specified in Sequest as variable modifications.

4.3.6. Criteria for protein identification (performed by the UMN Proteomic center)

Scaffold (v. Scaffold_4.7.3, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 97.0%

probability to achieve a False Discovery Rate (FDR) less than 1.0% by the Scaffold Local FDR algorithm. Protein identities were accepted if they could be established at greater than 99.0% probability to achieve an FDR less than 1.0% and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (414). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

4.3.7. Quantitative data analysis

Scaffold Q+ (v. Scaffold_4.7.3, Proteome Software Inc., Portland, OR) was used to quantitate Label Based Quantitation (iTRAQ) peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 97.0% probability to achieve an FDR less than 1.0% by the Scaffold Local FDR algorithm. Protein identities were accepted if they could be established at greater than 99.0% probability to achieve an FDR less than 1.0% and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (414). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Normalization was performed iteratively (across samples and spectra) on intensities, as described in Statistical Analysis of Relative Labeled Mass Spectrometry Data from Complex Samples Using ANOVA (415). Medians were used for averaging. Spectra data were log-transformed, pruned of those matched to multiple proteins and those missing a

reference value, and weighted by an adaptive intensity weighting algorithm. Of 41277 spectra in the experiment at the given thresholds, 38914 (94%) were included in quantitation. A total of 1435 proteins were identified.

For the global analysis, differentially expressed proteins in the different samples compared to the average of the pooled control samples were determined by Scaffold Q+ by applying a permutation test with significance threshold set at *p*-value < 0.05. Then, to obtain the fold change of each protein between every sample compared to Day 0, the fold change of the proteins in each sample (calculated by averaging the fold changes ratios for both quants) were divided by the average fold change ratio of the Day 0 sample (beads inoculation step), used as reference. The fold change ratios were then converted to $\log_2$ [$\log_2$ (fold change)].

4.3.8. Principal components analysis and hierarchical clustering

Principal components analysis (PCA) and two-way hierarchical clustering were performed using the JMP Pro 13.0.0 software (SAS, Cary, NC USA). The hierarchical clustering was performed using the Ward method and represented as a two-way clustering dendrogram using distance as scale.

4.3.9. Statistical analyses

The experiments were performed in duplicate, with each biological duplicate performed on a different day. For each biological duplicate, three different technical replicates were collected and mixed together for protein extraction. Statistical analysis among the protein expression levels in the different samples

was performed as described in Section 4.3.6. The *p*-values between the different sample groups described in Table 4.1 were determined using a two-tailed Student's t-test assuming equal variance for all experiments. Threshold for significance was set at $p \leq 0.05$.

4.4. Results

4.4.1. PCA analysis and hierarchical clustering

The initial global analysis in Scaffold Q+ identified 734 differentially expressed proteins (*p*-value < 0.05) between the pooled samples used as control and the 6 conditions tested (Day 0, after drying, after additional equilibration to a_w 0.11, after thermal treatment at 75°C at a_w 0.11, after additional exposure to a_w 1.0, and after thermal treatment at 75°C at a_w 1.0) (Appendix 4). For all 734 proteins, the expression fold change compared to Day 0 was calculated, as explained in the Materials and Methods. A primary principal component analysis (PCA) on the entire set of proteins resulted in a component 1 of 92.5% and a component 2 of 6.47% (Fig 4.1 A). The initial analysis revealed a clear separation between the "dry" samples (after drying, a_w 0.11, and a_w 0.11 thermally treated) and the "wet" samples (a_w 1.0 and a_w 1.0 thermally treated), but no separation was observed among the 734 proteins (Fig 4.1 B).

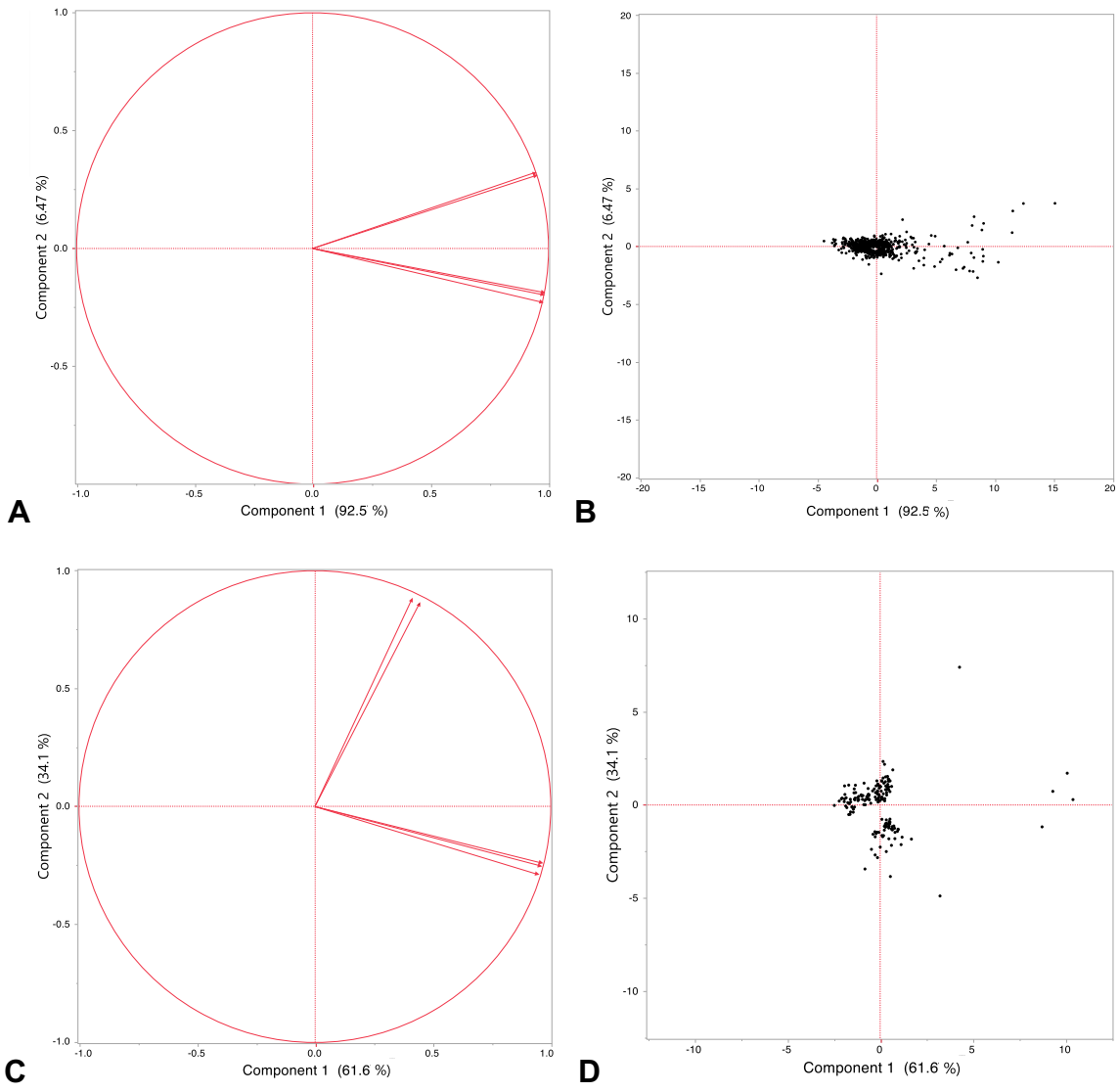
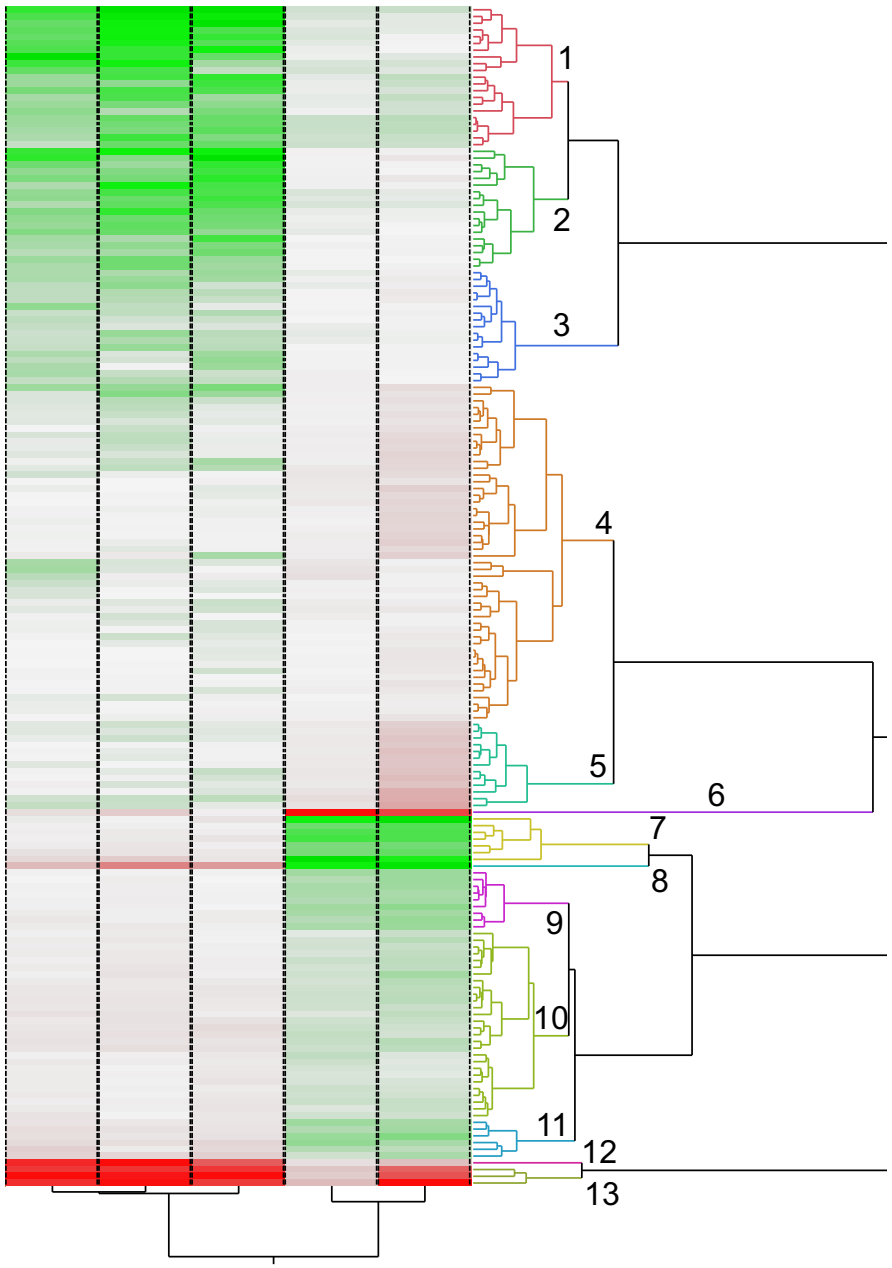


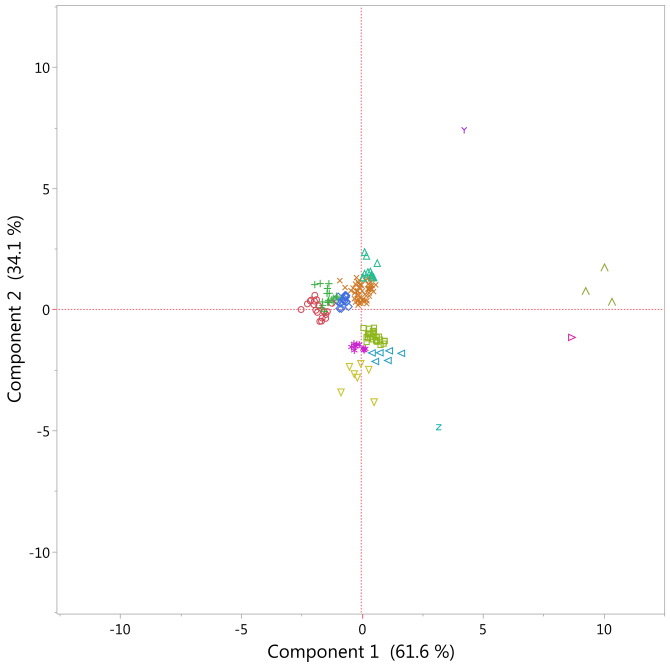
Fig 4.1. Two-dimensional PCA plots representing the distribution of the 5 different *Salmonella* samples based on differential protein analysis (A and C) and the differentially expressed proteins (B and D). First PCA plots showing (A) the distribution of the different samples (top to bottom: a_w 1.0, a_w 1.0 thermally treated, a_w 0.11, a_w 0.11 thermally treated, after drying) and (B) the distribution of the 734 differentially expressed proteins. Final PCA showing (C) the distribution of the different samples (top to bottom: a_w 1.0, a_w 1.0 thermally

treated, after drying, a_w 0.11, a_w 0.11 thermally treated) and the separations of the 175 proteins in two major groups and 6 outliers (D).

We selected the proteins contributing to the differences between conditions by first performing a hierarchical clustering (Appendix 5) with intracluster ordering based on the first principal component from the PCA. The data were selected by removing from the subsequent analyses those clusters of proteins that did not show variations between the two group of samples (“dry” and “wet”). We obtained a final number of 175 proteins (Appendix 4). A PCA (Fig 4.1 C) on this smaller set of proteins resulted in a better separation between the 2 major groups, “dry” and “wet”, with the first two components of 61.6% and 34.1%, respectively. Two clearly distinct sets of proteins and 6 outliers were distinguishable (Fig 4.1 D). A two-way hierarchical clustering performed on this group of 175 proteins identified 13 clusters in the two major groups “dry” and “wet” (Fig 4.2 A), which were separated in two groups and 4 outlier clusters in the PCA (Fig 4.2 B). The hierarchical clustering also showed that the proteomes of dried and low a_w samples clustered together and were separated from the high a_w samples (Fig 4.2 A).


1
2
3
4
5
6
7
8
9
10
11
12
13
After drying
a_w 0.11
a_w 0.11 thermal
a_w 1.0
a_w 1.0 thermal

A



B

Fig 4.2. Hierarchical clustering heat map of 175 proteins differentially expressed in *Salmonella* cells subjected to 5 different treatments (A) and PCA plot of the 13 clusters (B). The hierarchical clustering (A) shows a division between the "dry" samples on the left (after drying, a_w 0.11, and a_w 0.11 thermally treated) and the "wet" samples on the right (a_w 1.0 and a_w 1.0 thermally treated). Different colors and numbers indicate the 13 clusters identified. The expression level of the proteins is indicated by a green/red scale where green is low expression and red is high expression. The PCA plot (B) shows the distribution of the 13 clusters identified by hierarchical analysis (different colors and symbols) based on component 1 and 2.

4.4.2. Cluster analysis

Following hierarchical clustering, each of the 13 clusters was further characterized, and the clusters were divided into 2 major sets based on the difference between the 2 groups of samples, “dry” and “wet”, according to what was observed with the PCA and the two-way hierarchical analysis (Fig 4.3). All the clusters in which the protein expression levels for the “dry” samples were lower than the expression levels for the “wet” samples were included in the ‘L’ set, whereas all the clusters where the proteins expression levels were higher in the “dry” samples compared to the “wet” samples were assigned to the ‘H’ set.

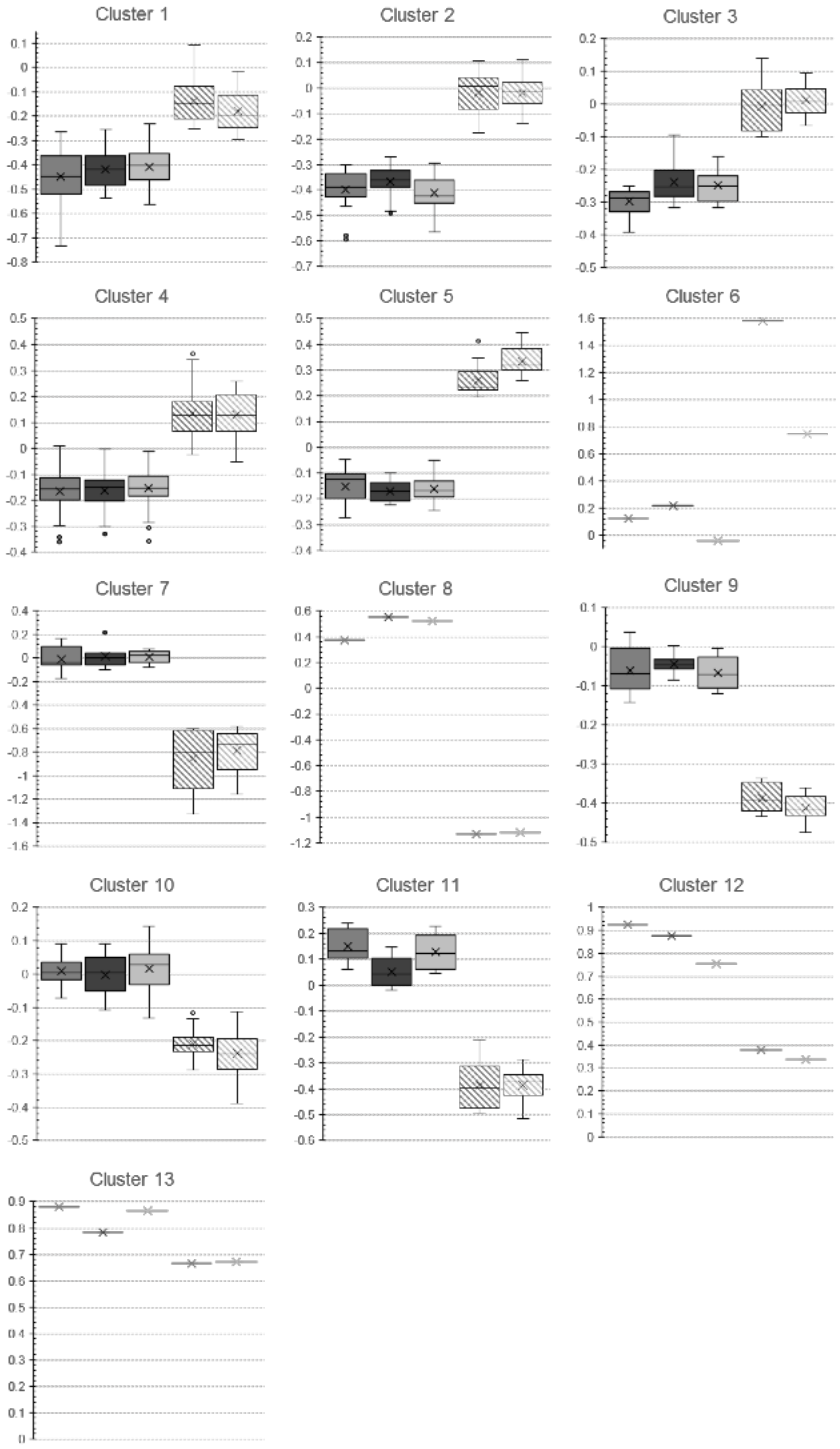


Fig 4.3. Differential protein expression levels of *Salmonella* cells distributed into clusters identified by two-way hierarchical clustering. The “dry” sample

group is divided in after drying (grey), a_w 0.11 (dark grey), and a_w 0.11 thermally treated (light grey), while the "wet" sample group is divided in a_w 1.0 (dark grey stripes) and a_w 1.0 thermally treated (light grey stripes). The protein expression levels are expressed as $\log_2$ (fold change) between each sample and the Day 0 sample. In each box, the x represents the mean and the horizontal line represents the median.

Set 'L' included 6 clusters (cluster 1 to 6), for a total of 120 proteins and 68.8% of the total (Table 4.2). The largest cluster was cluster 4, containing 50 proteins (28.6% of the total), while the smallest cluster was cluster 6 with only one protein (YcgM). This cluster was originally identified as an outlier in our PCA, because the expression fold changes between the after drying and Day 0, as well as between a_w 0.11 and Day 0 were positive [0.12 and 0.22 $\log_2$ (fold change), respectively] instead of being negative as in the rest of the set 'L'. In our analysis, however, this cluster has been included in set 'L', since the focus was to characterize the variations in protein expression patterns between the two main groups of samples, and, therefore, the specific fold change compared to Day 0 was relevant only for the comparison between the two sample groups. The *p*-values were calculated for each cluster in set 'L', as a statistical indication of the difference between the two sample groups. In set 'L', the *p*-values ranged from 2.7×10^{-86} for cluster 4, to 4.7×10^{-2} for cluster 6 (Table 4.2).

Table 4.2. Distribution of 175 differentially expressed proteins in *Salmonella* cells identified by hierarchical clustering analysis. Sets were identified based on a significantly lower (L) or higher (H) protein expression of the "dry" samples (dried, a_w 0.11, a_w 0.11 thermally treated) when compared to the "wet" samples (a_w 1.0, a_w 1.0 thermally treated). *p*-values are calculated for each cluster as indication of the significance of the differences observed between the "dry" and the "wet" sample groups.

Set	Cluster	Nº of proteins	% of total	*p*-value "dry" vs "wet"
L	1	21	12	$1.5x10^{-27}$
	2	18	10.3	$1.5x10^{-39}$
	3	17	9.7	$3.0x10^{-35}$
	4	50	28.6	$2.7x10^{-86}$
	5	13	7.4	$3.1x10^{-18}$
	6	1	0.6	$4.7x10^{-2}$
H	7	7	4	$4.4x10^{-16}$
	8	1	0.6	$2.0x10^{-4}$
	9	9	5.1	$6.2x10^{-29}$
	10	28	16	$7.8x10^{-52}$
	11	6	3.4	$6.4x10^{-16}$
	12	1	0.6	$5.0x10^{-3}$
	13	3	1.7	$1.0x10^{-3}$

Set 'H' included the remaining 7 clusters, all of which presented a higher protein expression level in the "dry" samples compared to the "wet" samples. The largest cluster was cluster 10, with 28 proteins (16% of the total), while the smallest were clusters 8, 12, and 13 (1, 1, and 3 proteins, respectively). These clusters were originally identified as outliers in our PCA. Cluster 8, although being characterized by higher expression levels for the "dry" samples compared

to the “wet” samples, presented the largest variation between the two sample groups [1.61 $\log_2$ (fold change)], with a mean $\log_2$ (fold change) of 0.48 and -1.13 for the “dry” and the “wet” samples, respectively. Clusters 12 and 13 were different from the other clusters in set ‘H’ because the fold change for both sample groups were positive, indicating a higher expression compared to Day 0, while the rest of the clusters had at least one sample group whose fold change was lower than Day 0 [negative $\log_2$ (fold change) values]. Also for set ‘L’, the *p*-values calculated between the two sample groups were all statistically significant, and ranged from 7.8×10^{-52} for cluster 10 to $5. \times 10^{-3}$ for cluster 12 (Table 4.2).

4.4.3. Differentially expressed proteins

The 175 proteins were functionally classified using the KEGG Orthology database to determine their role in the global cellular physiology. Of the 175 proteins, 97 were classified based on 5 functional groups; 1) metabolism, 2) genetic information processing, 3) environmental information processing, 4) cellular processes, and 5) virulence. The remaining 78 proteins could not be classified in any orthology group. As shown in Fig 4.4, the largest category was metabolism with 61 proteins, followed by genetic and environmental information processing (18 and 16 proteins, respectively). Some proteins were involved in more than one pathway and were therefore classified in multiple functional categories. For example, the flagellin proteins, FljB and FliC, were classified in the environmental information processing, cellular processes, and virulence categories.

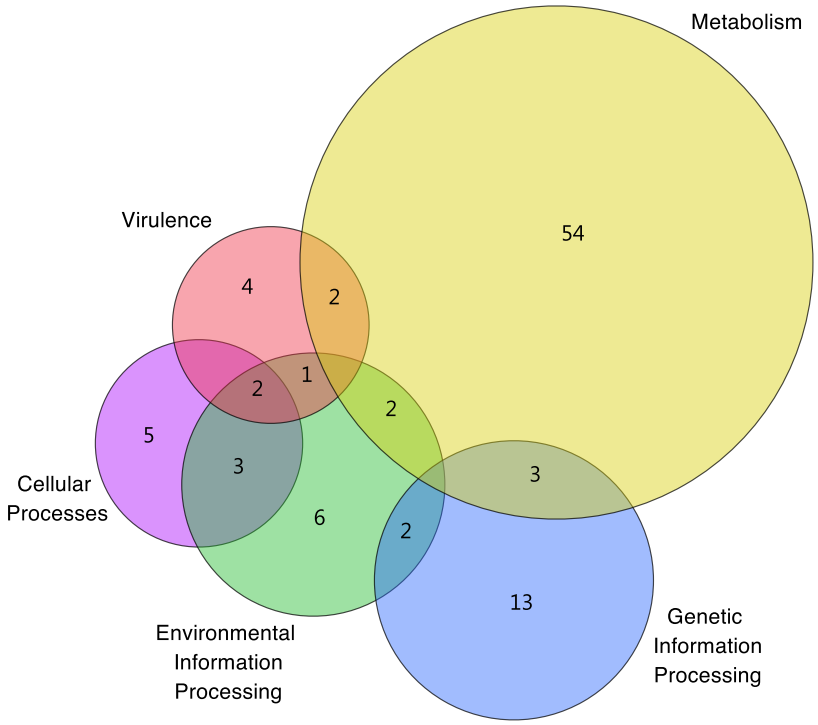


Fig 4.4. KEGG Orthology classes. Venn diagram representing the division in functional classes base on the KEGG Orthology database analysis of the 175 proteins differentially expressed in the 5 samples.

Table 4.3 comprises a selected group of proteins belonging to set 'L', that included proteins whose expression was higher in the a_w 1.0 group compared to the "dry' group (after drying and a_w 0.11 samples). Among these, multiple metabolic proteins were identified. In particular, TreA, a periplasmic trehalase that catalyzes the hydrolysis of trehalose into two molecules of glucose (331), as well as MogA, MoaB, and MoaC, which are involved in molybdenum cofactor biosynthesis (416). Many transporters were also more expressed in the "wet"

samples, including the copper-exporting ATPase CopA (417), then zinc/cadmium exporter ZntA (418), and some ABC transporters.

In set ‘L’, we also identified several flagellar components (FllgE, FlgF, FlgF, FlgH) and two flagellin components (FliC and FljB). Also, various membrane proteins and membrane protein transporters were more abundant in “wet” samples compared to “dry” samples. More specifically, BamA, BamB, and BamD, components of the outer membrane protein assembly complex Bam (419, 420), SecD and SecF, components of the Sec translocon, LolA and LolB, a chaperon and an outer membrane assembly protein, respectively, involved in the transport of lipopolysaccharides (LPS) to the outer membrane (421, 422), and LptD, required for LPS transport across the outer membrane (419, 423) were all significantly higher in expression under “wet” conditions when compared to “dry” conditions.

Proteins involved in replication, transcription, and translation were also identified in set ‘L’. Among these were TatD, a magnesium-dependent exonuclease involved in DNA degradation during apoptosis as well as in response to H_2O_2-induced DNA repair (424), Tus, a DNA-binding protein part of the Tus-TerB DNA replication termination complex (425), and RraA, a ribonuclease regulator (426). We also identified proteins required for tRNA biogenesis (e.g. SerS, YihZ, and GltX).

Other proteins identified as stress-response proteins were found to be more abundant in set ‘L’. Among these, GorA, a glutathione oxidoreductase

(427), and Dps, a ferritin-like protein that protects DNA from damage under starvation and long-term stationary phase (428), were identified. Both these genes are involved in oxidative and starvation stress-response. Other proteins of note that were differentially expressed from set 'L' include, HtpX, a zinc-dependent inner membrane endoprotease under the control of CpxR-CpxA (429, 430), the stringent starvation protein SspA (431, 432), and the sensor protein BasS, part of the PmrAB two-component system (433).

Table 4.3. Selected group of proteins with greater expression level patterns in *Salmonella* from "wet" samples compared to "dry" samples.

	Accession number	Protein description	Name	Cluster
Metabolism				
	A0A0F6B297_SALT1	Periplasmic trehalase	TreA	4
	A0A0F6AWC4_SALT1	Molybdenum cofactor biosynthesis protein	MogA	4
	MOAC_SALTY	Molybdenum cofactor biosynthesis protein	MoaC	4
	A0A0F6AYV4_SALT1	Molybdenum cofactor biosynthesis protein	MoaB	5
Transporters				
	COPA_SALTY	Copper-exporting P-type ATPase A	CopA	1
	A0A0F6BA72_SALT1	Maltose ABC transporter periplasmic protein	MalE	2
	A0A0F6B7A6_SALT1	Putative ABC transporter ATP-binding protein	YhbG	4
	A0A0F6AZ15_SALT1	Putative ABC transporter periplasmic binding protein	YliB	4
	Q8ZLE5_SALTY	zinc/cadmium transporting protein	ZntA	4
Replication, transcription, and translation				

	TATD_SALTY	3'-5' ssDNA/RNA exonuclease	TatD	1
	TUS_SALTY	DNA replication terminus site-binding protein	Tus	3
	A0A0F6AZA8_SALT1	Serine-tRNA ligase	SerS	3
	A0A0F6B9J7_SALT1	D-aminoacyl-tRNA deacylase	YihZ	3
	A0A0F6B4G4_SALT1	Glutamyl-tRNA synthetase	GltX	4
	RRAA_SALTY	Regulator of ribonuclease activity A	RraA	5
Stress response				
	A0A0F6B7D2_SALT1	Stringent starvation protein A	SspA	1
	Q7CPU8_SALTY	oxidative stress defense protein	YggE	1
	Q8ZLD4_SALTY	Glutathione oxidoreductase	GorA	2
	A0A0F6B2F5_SALT1	zinc-dependent endoprotease	HtpX	3
	BASS_SALTY	Sensor protein part of the PmrA/PmrB system	BasS	4
	USPG_SALTY	Universal stress protein G	UspG	4
	DPS_SALTY	starvation/stationary phase protein	Dps	4
Motility				
	FLGE_SALTY	Flagellar hook protein	FlgE	4
	FLGG_SALTY	Flagellar basal-body rod protein	FlgG	4
	FLJB_SALTY	Phase 2 flagellin	FljB	4
	A0A0F6B013_SALT1	Flagellar basal body protein	FlgF	5
	A0A0F6B015_SALT1	Flagellar L-ring protein	FlgH	5
	FLIC_SALTY	Flagellin	FliC	5
Membrane and protein export				

	A0A0F6AZA5_SALT1	Outer-membrane lipoprotein carrier protein	LolA	1
	A0A0F6B274_SALT1	Outer-membrane lipoprotein	LolB	1
	A0A0F6AWM8_SALT1	LPS-assembly protein	LptD	5
	A0A0F6B4T1_SALT1	Outer membrane protein assembly factor BamB	YfgL	2
	Q8ZMW8_SALTY	Outer membrane protein assembly factor BamD	YfiO	4
	A0A0F6AX28_SALT1	Outer membrane protein assembly factor BamA	YaeT	4
	Q8ZRD7_SALTY	Protein translocase subunit SecD	SecD	4
	A0A0F6AXN1_SALT1	membrane protein translocase subunit SecF	SecF	4

Set ‘H’, which included proteins whose expression was higher in “dry” samples compared to the “wet” samples (Table 4.4) presented a very different proteomic profile. Among these were proteins belonging to metabolic pathways, e.g. GlpX, fructose-1,6-bisphosphatase involved in gluconeogenesis (434), AdhE, alcohol dehydrogenase part of fermentative pathways (435), and AcnA, aconitase part of the Kreb cycle (436), as well as proteins involved in tRNA charging of different aminoacids, e.g. TrpS for tryptophan, GlyS for glycine, ThrS for threonine, and AlaS for alanine. Also, proteins involved in DNA replication and repair (DnaJ, UvrD), replication regulation (SeqA), transcriptional regulation (StpA), and degradation of mRNAs (RhlB) were also more abundant in the “dry” samples, together with several ribosomal proteins, including the 50S ribosomal proteins L2 (RplB), L25 (RplY), L31 (RpmE), and L34 (RpmH) and the 30S

ribosomal proteins S3 (RpsC) and S12 (RpsL), as well as the virulence factor SipA, a SPI-1 encoded effector protein.

Table 4.4. Selected group of proteins with greater expression level patterns in *Salmonella* cells from "dry" samples compared to "wet" samples.

	Accession number	Protein description	Name	Cluster
Metabolism				
	A0A0F6B9R6_SALT1	Fructose-1,6-bisphosphatase	GlpX	10
	Q8ZP45_SALTY	Aldehyde-alcohol dehydrogenase	AdhE	10
	A0A0F6B200_SALT1	Aconitate hydratase	AcnA	13
Replication, transcription, translation, and post-translational regulation				
	A0A0F6AZM9_SALT1	Ribosome modulation factor	Rmf	7
	A0A0F6B5K2_SALT1	DNA-binding protein	StpA	7
	SYW_SALTY	Tryptophan-tRNA ligase	TrpS	7
	A0A0F6B282_SALT1	Ribosome-binding ATPase	YchF	9
	A0A0F6B771_SALT1	Translation initiation factor IF-2	InfB	9
	DNAJ_SALTY	Chaperone protein DnaJ	DnaJ	9
	SYGB_SALTY	Glycine-tRNA ligase	GlyS	9
	Q8ZMN7_SALTY	DNA helicase	STM2767	10
	SYT_SALTY	Threonine-tRNA ligase	ThrS	10
	UVRD_SALTY	DNA helicase II	UvrD	10
	Q8ZLJ1_SALTY	Putative RNase R	YhgF	10

	SYA_SALTY	Alanine-tRNA ligase	AlaS	10
	A0A0F6AYJ6_SALT1	Negative modulator of initiation of replication	SeqA	11
	RHLB_SALTY	ATP-dependent RNA helicase RhlB	RhlB	11
Ribosomal				
	A0A0F6B9S9_SALT1	50S ribosomal protein L31	RpmE	7
	RS12_SALTY	30S ribosomal protein S12	RpsL	7
	RL34_SALTY	50S ribosomal protein L34	RpmH	8
	A0A0F6B7N0_SALT1	30S ribosomal protein S3	RpsC	11
	A0A0F6B7N3_SALT1	50S ribosomal protein L2	RplB	11
	RL25_SALTY	50S ribosomal protein L25	RplY	11
Virulence				
	A0A0F6B5V0_SALT1	Secreted effector protein	SipA	10

4.5. Discussion

In this study, we present the results of a global proteomic comparative analysis by iTRAQ of *Salmonella* cells dried, exposed to high and low a_w, and thermally treated. While the effects of desiccation on *Salmonella* have been studied using transcriptomic techniques by several groups (239, 279, 280, 286, 315), to our knowledge, this the first time that a global proteomic analysis has been performed for desiccated and thermally treated *Salmonella*. Our results showed that pre-adaptation to desiccation and low a_w is responsible for *Salmonella* tolerance to heat treatment. Indeed, large differences in protein

expression patterns were mainly observed between the two main sets of samples, “dry” and “wet”, whereas differences between thermally treated and not treated samples were small and limited to fewer proteins at both a_w (Appendix 6).

It is important to remember that both “dry” and “wet” samples underwent a drying step before being equilibrated to the respective a_w. This means that even the “wet” sample cells had to adapt to desiccation, before being re-exposed to moisture. This implies that most likely we observed the effect on the proteome of the rehydration of the desiccated cells. This is corroborated by the profiles of the protein expression among the 5 samples. In fact, protein abundances were very similar between the dried and the a_w 0.11 samples (both thermally treated and not), while very different from all the a_w 1.0 samples (both thermally treated and not). Comparisons of all the conditions tested to Day 0 showed that protein levels were generally lower in all the conditions than at Day 0. This is not surprising, since the cells at Day 0 were collected after overnight growth in a rich medium. Even though the cells were in stationary phase and nutrient starved, they still had some source of nutrients generated by lysated cells and somewhat homogenously dispersed by diffusion through the liquid medium. Conversely, the cells on the beads only had the local nutrients available through adjacent cells secretions and lysis. As a result, the metabolic rates and protein synthesis of the Day 0 cells were likely higher than in the other samples.

The differential expression of proteins involved in DNA stabilization, regulation of replication, transcription, and regulation of translation between the

"dry" and "wet" sets indicates that the cells in both "dry" and "wet" conditions need to strictly regulate the rate of replication/ growth rate, and cell division. Higher abundance of DNA replication and repair proteins, as well as transcriptional and translational regulators in the "dry" samples confirmed what we observed in our transcriptomic analysis of samples at a_w 0.11 and 1.0 (see Chapter 2). One of the effects of desiccation and low a_w is damage to DNA molecules, e.g. covalent modifications and breaks in the double helix (316), and the up-regulation of DNA repair genes which has been described in many microorganisms, including *Deinococcus radiodurans* (317) and *Bradyrhizobium japonicum* (318).

In our experiments, we observed higher expression of SeqA in "dry" samples compared to "wet" samples. SeqA was first discovered as a DNA-binding protein able to bind to the hemimethylated origin of replication *oriC* in *E. coli* thus sequestering the DNA site from DnaA, and preventing re-initiation of replication (437-440). This might indicate that re-initiation of DNA replication is prevented. In *Salmonella*, *seqA*$^-$ mutants have shown higher sensitivity to H_2O_2 and bile salts (441), and *in vitro* assays suggest that mutations in this gene affect *Salmonella* pathogenicity, decreasing its adhesion and invasion abilities (442).

Proteins involved in the biogenesis of tRNAs were found in both sets of proteins, suggesting that regulation of tRNA could be fundamental for adaptation to different environmental conditions, as also suggested by our analysis at the

transcriptome level (see Chapter 2). In that case, genes encoding for various tRNAs were found to be up-regulated at low a_w.

As expected, a change in metabolism was observed between the two sets. TreA, responsible for the hydrolysis of trehalose into two molecules of glucose in the periplasm (443), was found to be more abundant in “wet” samples than in “dry” samples. This finding contradicts what previously described by Li *et al* (280). In their study, Li *et al.* detected an increase in the expressionof *treA* in *S. enterica* serovar Typhimurium LT2 desiccated for 2 hours on filter paper at 11% ERH compared to cells spotted on filters and not desiccated (280). One possible explanation for the different result observed could be the time of the observation, as well as the conditions of exposure. While we analyzed samples after a long-term exposure to low a_w, Li *et al*. observed the changes in the first hours of adaptation. More importantly, the re-suspension of bacterial cells in 0.1 M PBS before inoculation on filters could be triggering the activation of osmotic response during desiccation due to the increase in solute concentration while water is lost, rather than being a direct effect of lowering the a_w, as already suggested for isotonic solutions (286). It is indeed known that, under osmotic stress, cells activate the cytoplasmic synthesis of trehalose (*otsAB*) as osmoprotectant, while the periplasmic trehalase TreA is activated in order to hydrolize trehalose into glucose to be imported through the PTS system and be used for glycolysis (444-446).

Trehalose accumulation is a well-known component of the desiccation defense mechanism. Trehalose works both as an osmoprotectant induced by osmotic stress, and as a membrane stabilizer by replacing water clathrates around macromolecules, thus preventing desiccation damage (258, 447). This last function is thought to be due to the structure of the α,α-(1→1) glycosidic bond between the two molecules of glucose, which allows this molecule to form clam shell structures, thus facilitating interactions between the sugar and the lipid headgroups of the membrane (448). In our case, we hypothesize that when equilibrating to high a_w after desiccation, the membranes return to their hydrated state, and trehalose accumulation in the phospholipid layer is no longer necessary. This trehalose is then released in the periplasm, where TreA hydrolizes it into glucose. The absence of higher expression for proteins involved in trehalose synthesis in “dry” samples was in agreement with what we observed in the transcriptome of the cells equilibrated for 4 days at a_w 0.11 (see Chapter 2).

Besides, at high a_w, when the stress from desiccation ceases and the metabolic rate can increase, the cell needs to activate catabolism to produce ATPs: under these conditions, accumulating trehalose would waste an important energy and carbon resource. Supporting this hypothesis, we found that GlpX, enzyme converting fructose 1,6-bisphosphate into fructofuranose 6-phosphate in gluconeogenesis (434), was less expressed in the “wet” than in the “dry” samples.

A correct assembly and transport of Outer Membrane Proteins (OMPs) is required for membrane integrity, and therefore for cell division. The presence of membrane protein transporters and assembly complexes at higher level in “wet” samples compared to dry samples supports the idea that cells exposed to a_w 1.0 are more metabolically active and have activated a series of responses to favor cellular growth and replication. Higher amounts of YaeT (BamA), YfgL (BamB), and YfiO (BamD) were detected in “wet’ samples compared to “dry” samples. These proteins, together with NlpB (BamC), whose expression was not significantly different between the two groups of samples, and SmpA (BamE), which was not identified in any sample, form the Bam complex, which is required for the assembly and the transport of outer membrane proteins (OMPs) in *Salmonella* (449, 450). The higher abundance of three out of four proteins of the Bam complex in “wet” samples indicates the importance of the entire Bam complex in ensuring a correct membrane assembly during adaptation and survival at high a_w. Similarly, HtpX was found to be more abundant in samples equilibrated at a_w 1.0 (but not in a_w 1.0 thermally treated). HtpX is a membrane protein with proteolytic activity (429), and is involved in the membrane-protein degradation on the cytoplasmic side of the inner membrane, and is under the control of the CpxA-CpxR regulon in response to the accumulation of misfolded proteins under stress conditions (430).

At a_w 1.0, expression of flagella was higher than in dry conditions. Suppression of flagella expression has been previously described for *Salmonella*

under desiccating conditions (279), as well as for other microorganisms, such as *Bradyrhizobium japonicum* (451). It has been suggested that down-regulation of chemotaxis and motility during prolonged desiccation is associated with the cell need to preserve energy by shutting down dispensable functions and allowing the redirection of ATP towards essential cellular functions (405, 452). The general slowdown in cell growth and metabolic rates is reflected by the decrease in protein synthesis, as we observed higher expression of RhlB in “dry’ samples. Together with PNPase Pnp and the enolase Eno, whose expressions did not change between the two groups of samples, this protein is part of the degradosome (453-455) and takes part in the modulation of the level of transcripts available for translation, and therefore the amount of proteins produced (456). Conversely, RraA, a regulator of the ribonuclease activity of RNase E (Rne) was found more expressed in “wet” samples. RraA interacts with both RhlB and RNase E on the degradosome (456, 457), and inhibits the RNase activity of RNase E (426, 456), for which we did not observe any significant change in epression between the two groups of samples.

Surprisingly, various ribosomal proteins, including 30S and 50S subunit proteins, were more abundant in “dry” than in “wet” samples. The rate of cellular growth is strictly related to the rate of proteins synthesis (458). Since the rate of proteins synthesis per ribosomal unit has been shown to be constant and independent of growth rate (459), it has been suggested that the number of ribosomal units is what determines the rate of proteins synthesis (459-463).

Considering that the growth rate is almost zero in desiccating conditions, we would expect less synthesis of ribosomal units, and therefore less ribosomal proteins. Protein degradation with the purpose of energy recycle is mainly under the activity of 3 degradation systems: ClpXP, Lon, and ClpAP (464), and none of these proteins was differentially expressed in “dry” samples compared to “wet” samples. The role of ribosomal proteins in extra-ribosomal functions has been partially characterized in eukaryotic cells (271, 273, 465), and in *E. coli* the ribosomal protein L4 has been shown to bind RNAse E and, consequentially, modulate mRNA composition, in response to environmental stresses (466). A very intriguing possibility is that ribosomal proteins might play a specific role in modulating the adaptation to low a_w through specific extra-ribosomal functions, and therefore their expression level could be regulated independently from the protein synthesis rate required for cellular growth. An alternative explanation is that ribosomal proteins might be degraded at a higher rate in “wet” cells to re-cycle aminoacids for synthesis of other essential proteins. This hypothesis is supported by our data showing lower expression of ribosomal proteins in both “dry” and “wet” samples compared to Day 0. However, this explanation conflicts with our observation of a higher abundance of the degradosome component RhlB in “dry” samples compared to “wet” and of the higher abundance of RraA in “wet” samples compared to “dry”, suggesting a higher protein degradation in “dry” samples.

Our analysis of the *Salmonella* transcriptome at low a_w (see Chapter 2) detected the up-regulation of six virulence-related genes (*sscA*, *sseA*, *sopD*, *sseD*, *mgtC*, *mviN*). The importance of two of them, *sopD* and *sseD*, in survival to desiccation and low a_w was confirmed by specific *ad hoc* mutants. Similarly, in the analysis of the proteome, we found higher expression in "dry" samples compared to "wet" samples of the virulence protein SipA, a SPI-1 T3SS secreted effector that induces the uptake of *Salmonella* cells by the host cells by stabilizing the cytoskeleton actin filaments (467). SipA and SopD are co-secreted by the SPI-1 T3SS and have correlated function in promoting host cells invasion and uptake, together with other 4 effectors, SopA, SopB, SopE, and SopE2 (468, 469). SseD is also part of the T3SS injection mechanism (332). Our observation partially supports the transcriptome and mutants' analyses (see Chapter 2), in which we revealed a role of the two virulence genes *sopD* and *sseD* in *Salmonella*'s desiccation survival, and might also indicate that SPI-T3SS and the related effectors are involved in response to desiccation and low a_w conditions, a role that has yet to be described.

More stress response proteins (e.g. SspA, GorA, Dps, BasS) were found in set 'L' compared to 'H', indicating that the adaptation to moisture after being dried induces a general multiple-stress response system. This is very interesting, and partially unexpected, since many studies in literature report the activation of diverse stress-response systems when the cells undergo desiccation (279, 280, 286, 315, 407), which lead us to expect higher expression of stress-response

related proteins in “dry” samples compared to “wet” samples. GorA and Dps were among the stress-response proteins identified in set L. In *E. coli*, both these two proteins are part of the OxyR regulon, in response to oxidative stress induced by H_2O_2 during exponential phase (427, 470). During stationary phase the expression is controlled by RpoS (471), although for GorA the control could be an indirect effect (471). Dps has a dual function of DNA protection: as a regulator by its DNA binding activity, and as a chelator as a ferritin-like protein. Dps binds Fe(II) and facilitates the oxidation of Fe(II) by H_2O_2 by sequestering H_2O_2 and Fe(II) thus avoiding the hydroxyl radical formation by Fenton reaction (428). The higher abundance of this proteins in “wet” samples could be an indication that rehydration in aerobic environment causes the formation of ROS. In particular, rehydration might facilitate the spontaneous dismutation of O_2^-, by-product of aerobic respiration, into H_2O_2. O_2^- is also converted to H_2O_2 by superoxidodismutase SOD (372). Two SODs, SodA, a manganese-dependent SOD, and SodC1, a zinc- and copper-dependent SOD, were part of the 734 proteins initially identified, but their expression was not different between the two groups of samples. O_2^- also reduces Fe(III) to Fe(II), which then reacts with H_2O_2 in the Fenton reaction, producing hydroxyl radical $OH^{\cdot}$ (372). Therefore, accumulation of O_2^- leads to accumulation of H_2O_2 and $OH^{\cdot}$(372). These data taken together could explain why the Fe(II) chelating protein Dps was more abundant in “wet” samples compared to “dry” samples.

The stringent starvation protein SspA was more abundant in “wet” samples than in “dry” samples. In *E. coli*, SspA, together with SspB, whose expression was not different between the 2 groups of samples, has been found to act as a global regulator that activates cellular defense systems in response to nutrient starvation through inhibition of the global transcriptomic repressor H-NS (432), whose expression did not variate between the “dry” and the “wet” samples. This suggested that long-term starvation plays an important role in the regulation of the proteomic profile observed in cells after a week of high a_w equilibration.

It is clear that the response systems to starvation and oxidative stress overlap significantly, and that survival at higher a_w in growth conditions that are not optimal requires a long-lasting response (considering that the samples were collected after one week of equilibration to a_w 1.0). The presence of these proteins at higher levels in “wet” cells compared to “dry” cells indicates that cells re-exposed to high a_w are subjected to more cellular stress than adapted “dry” cells. “Wet” cells were in an environment that allowed for chemical and enzymatic reactions, differently from “dry” cells, that were at very low a_w (a_w lower than 0.6), at which enzymatic reactions were dramatically slowed down, if not completely interrupted (472). Although the a_w is high, the growth conditions were far from being optimal for growth and survival, considering the long period of starvation to which they were exposed during the equilibration. It is possible that the contrast between the signal to grow and replicate, deriving from exposure to higher a_w, and the stressed metabolic state due to the lack of nutrients preventing the *de*

novo synthesis of proteins, resulted in the activation of a series of global stress response systems.

Our study is the first global proteomic analysis of both desiccated and thermally treated *Salmonella* cells performed using the iTRAQ method. Our analysis clearly showed that pre-adaptation to desiccation is fundamental for developing thermal tolerance, as the cellular proteomic profile between non-treated and thermally treated samples did not change. The analysis of the protein expression patterns clearly revealed that once dried, *Salmonella* cells do not have major changes in proteomic expression when equilibrated to low a_w and thermally treated, while a major cell adjustment is required to re-adapt to high a_w conditions.

5. Summary and Conclusions

The overall goal of this study was to characterize physiological and molecular mechanisms that contribute to *Salmonella*'s ability to survive desiccation and develop thermal tolerance. This thesis is composed of five chapters. Chapter 1 provided the introductory information, presented up-to-date knowledge on the subject, stated the problem, the null hypotheses, and the study's objectives. Chapters 2, 3, and 4 are studies with different objectives, and are divided into four main sections: introduction, materials and methods, results, and discussion. In chapter 5, Summary and Conclusions, specific outcomes of every research chapter are highlighted.

Chapter 2: The objective of this study was to determine the genetic components involved in *Salmonella*'s adaptation to low a_w. We performed a global transcriptome analysis of *S. enterica* equilibrated to two different a_w, 0.11 and 1.0. Our analysis revealed that 290 genes were up-regulated at low a_w compared to high a_w. Many of these genes were involved in metabolic pathways, transporter regulation, DNA replication and repair, transcription and translation, and virulence. We focused our attention on two virulence genes, *sopD* and *sseD*, and constructed knock-out mutants. Both *sopD* and *sseD* mutants exhibited impaired ability to survive desiccation, as well as equilibration to a_w 0.11, compared to the wild-type strain. Moreover, scanning electron microscopy

showed that both mutants displayed a different morphology from the wild-type and were affected in their ability to produce extracellular matrix in desiccating conditions.

Chapter 3: The objective of this study was to characterize the effect of growth conditions and matrices on desiccation survival and development of thermal tolerance of *Salmonella*. *Salmonella* grown in LBglc and inoculated on toasted oat cereal (TOC) had higher recovery after desiccation and exposure to a_w 0.11, as well as greater thermal tolerance, than when inoculated on micro glass beads. The presence of chelating agents EDTA and dipyridyl during growth did not affect *Salmonella*'s desiccation and a_w 0.11 survival on TOC, nor its thermal tolerance. When inoculated on glass beads, *Salmonella* grown in LBglc displayed higher recovery after desiccation and equilibration to a_w 0.11 compared to cells grown in M9, but the thermal tolerance was lower than M9-grown cells. Differences in production of extracellular matrix after equilibration to a_w 0.11 and thermal treatment were observed between LBglc- and M9-grown cells. Additionally, cells grown on glass beads as biofilm presented higher thermal tolerance at a_w 0.11 than cells inoculated on glass beads.

Chapter 4: The objective of this study was to determine the changes in the global proteomic profile of *Salmonella* in response to desiccation and thermal treatment. We used the multiplex iTRAQ technique to analyze the proteome of

Salmonella dried, equilibrated to a_w 0.11 and 1.0, and thermally treated. Our analysis identified 175 proteins as the main source of the variation observed among the different treatment's proteomes. The majority of these proteins were involved in DNA replication and repair, regulation of transcription and translation, and metabolic pathways. We also observed higher abundance of motility proteins, membrane and export proteins, as well as stress-response proteins in "wet" samples compared to "dry" samples, while ribosomal proteins were more abundant in "dry" samples. Our analysis determined that the main variation in proteomic profiles was between the "dry" samples and the "wet" samples, while only small variations were observed between the thermally treated and non-treated samples. Our data indicated that adaptation to dry conditions is essential for development of thermal tolerance, while the reversion into a wet state causes the loss of thermal tolerance, and the activation of multiple stress-response systems

In conclusion, this work demonstrated that many factors, including growth conditions, are involved in determining *Salmonella*'s adaptation to desiccating conditions and thermal tolerance. More importantly, it determined that adaptation to desiccation is the cross-protection mechanism that allows thermal tolance. This adaptation requires regulation at genetic, proteomic, and physiological level as well as preservation of dry conditions, which are essential for the cell to

maintain a physiological equilibrium at which sensitivity to thermal treatment is reduced.

Future work should aim to further characterize the role of specific components of the adaptation. In particular, it will be interesting to:

1. Investigate the regulatory role of *sopD* and *sseD* genes in the adaptation to desiccation, possibly performing a transcriptomical analysis of the mutants under desiccating condtions;

2. Characterize the role of other SPI-1 and SPI-2 virulence genes in desiccation survival;

3. Evaluate the effect of oxidative stress due to ROS formation on desiccation and thermal tolerance by conducting desiccation experiments under anaerobic conditions;

4. Identify the role of specific proteins involved in desiccation adaptation.

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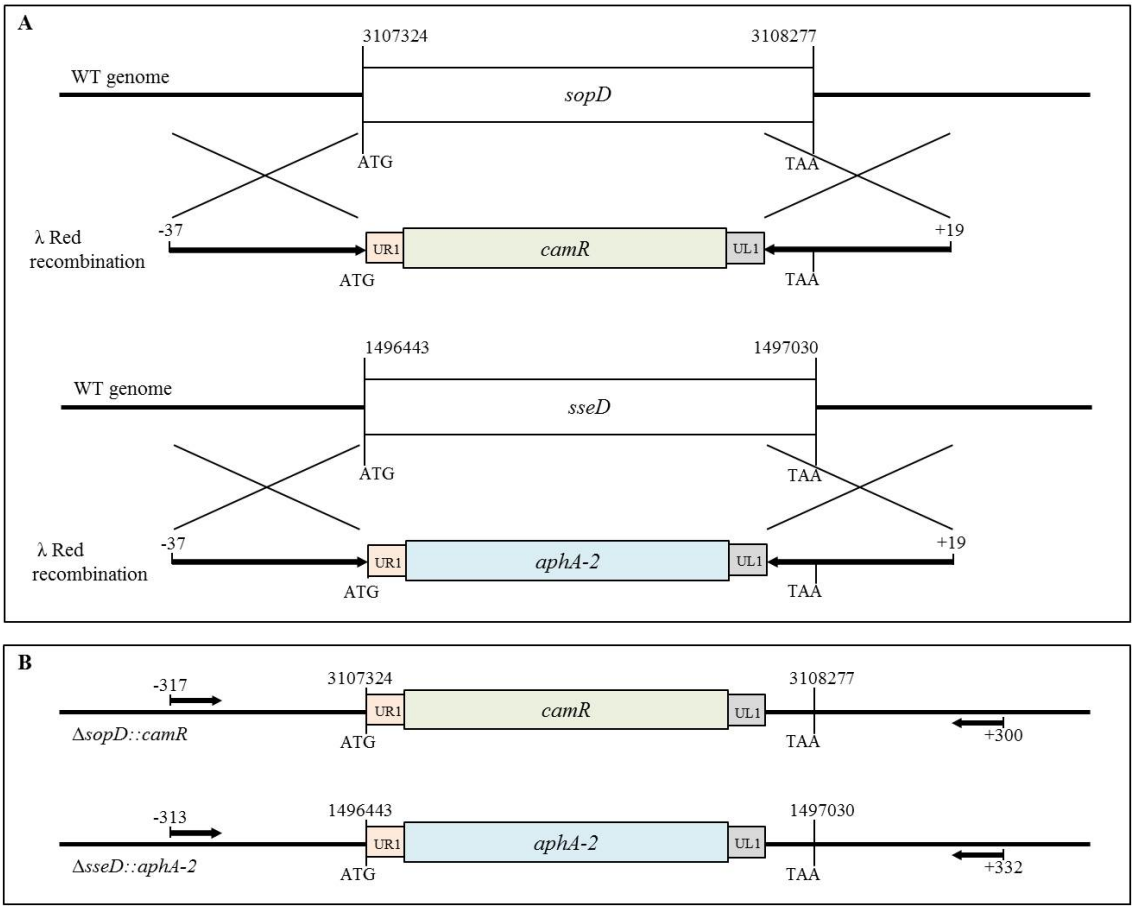
Appendices

Appendix 1. Schematic representation of the mutants and of the genomic regions amplified for PCR verification.

Schematic drawing of the *S. enterica* serovar Typhimurium wild-type *sopD* and *sseD* gene knock-out mutations with the chloramphenicol resistance cassette and the kanamycin resistance cassette, respectively (A). The sites of λ Red-mediated homologous recombination are indicated with crossing lines, while the primers used for the creation of the cassette are indicated with arrows. UL1 and UR1 are the universal caps part of the drug-cassette kit by Dr. Roth Laboratory (University of California at Davis, Davis, CA). Fig B shows the collocation of the primers used in the PCR reaction for the verification of Δ*sopD* and Δ*sseD* mutants of *S. enterica* serovar Typhimurium.



Appendix 2. Primers used for PCR verification and sequencing of the Δ*sopD* and Δ*sseD* mutants.

Gene	Primer ID	Primer Sequence (5'-3')	Direction
sopD	*sopD*-350verifFW	CTTCAGAAATATTTACCCCACG	Forward
	sopD+350verifRV	GGCGTGTTTAAAGTGCTACC	Reverse
sseD	*sseD*-350verifFW	GAGGGATTGTTCATTTAAAGGC	Forward
	sseD+350verifRV	CAGGATGCGCAATAATTTCC	Reverse

Appendix 3. List of genes differentially expressed (more than 2-fold change) in *S. enterica* serovar Typhimurium wild-type equilibrated to a_w 0.11 on filters.

Down-regulated genes			
Locus	**Gene name**	**Function**	**Fold change**
STM2970	*sdaC*	serine transport protein	-14.6
STM3975	*tatC*	twin-arginine protein translocation system subunit TatC	-13.1
STM0579	*ybdF*	hypothetical protein	-13.1
STM2256	*napB*	citrate reductase cytochrome c-type subunit	-13.1
STM1455	*ydgP*	electron transport complex protein RnfG	-12.4
STM4385	*ptxA*	PTS system L-ascorbate-specific transporter subunit IIA	-11.7
STM3473	*yhfC*	hypothetical protein	-11.7
STM1007	STM1007	hypothetical protein	-11.7
STM1045	STM1045	minor tail protein	-10.2
STM3665	*avtA*	valine--pyruvate transaminase	-10.2
STM2163	*yehX*	ABC-type proline/glycine betaine transport system ATPase component	-10.2
STM4557	*holD*	DNA polymerase III subunit psi	-10.2
STM0302	*safD*	fimbrial subunit	-8.8
STM0271	STM0271	hypothetical protein	-8.8
STM0813	*ybhP*	hypothetical protein	-8.8
STM0696	*ybfF*	hypothetical protein	-8.0
STM3054	*gcvH*	glycine cleavage system protein H	-8.0
STM0761	STM0761	fumarate hydratase	-7.3
STM4412	STM4412	permease	-7.3
STM1619	STM1619	cryptic aminoglycoside resistance gene	-7.3
STM1982	*rcsA*	colanic acid capsular biosynthesis activation protein A	-7.3
STM3930	*yifK*	transporter	-7.3
STM2799	*stpA*	DNA binding protein	-7.3
STM3830	*dgoR*	galactonate operon transcriptional repressor	-7.3
STM2832	*srlA*	glucitol/sorbitol-specific enzyme IIC component	-7.3
STM4019	*yihQ*	alpha-glucosidase	-7.3
STM3454	*slyX*	hypothetical protein	-6.8
STM2221	*bcr*	bicyclomycin/multidrug efflux system protein	-6.8
STM3378	STM3378	sulfite oxidase subunit YedZ	-6.8
STM3153	*yqhA*	hypothetical protein	-6.6
STM0361	STM0361	cytochrome BD2 subunit II	-6.3

STM2104	*cpsG*	phosphomannomutase	-6.3
STM1575	STM1575	transcriptional regulator	-6.3
STM0984	*msbA*	lipid transporter ATP-binding/permease	-6.3
STM1369	*sufA*	iron-sulfur cluster assembly scaffold protein	-6.1
STM2927	*surE*	stationary phase survival protein SurE	-5.8
STM3658	*yiaH*	inner membrane protein	-5.8
STM2106	*wcaI*	glycosyl transferase family protein	-5.8
STM3720	*yibR*	inner membrane protein	-5.8
STM2546	*suhB*	inositol monophosphatase	-5.8
STM0658	*ybeV*	molecular chaperone	-5.8
STM4104	STM4104	5'-nucleotidase	-5.8
STM0875	*rimK*	ribosomal protein S6 modification protein	-5.8
STM1675	STM1675	oxidoreductase	-5.8
STM1545	STM1545	multidrug efflux protein	-5.8
STM4566	*yjjI*	hypothetical protein	-5.8
STM1346	*ydiE*	hypothetical protein	-5.8
STM1247	STM1247	tRNA-Arg	-5.8
STM0435	*yajQ*	nucleotide-binding protein	-5.8
STM0432	*phnX*	phosphonoacetaldehyde hydrolase	-5.8
STM1471	*rstB*	sensor protein RstB	-5.8
STM1755	*ychJ*	hypothetical protein	-5.6
STM2023	*cbiM*	cobalt transport protein CbiM	-5.4
STM0206	*btuF*	vitamin B12-transporter protein BtuF	-5.1
STM3329	*yhcC*	FeS oxidoreductase	-5.1
STM1456	*rnfD*	electron transport complex protein RnfD	-5.1
STM3101	*yggT*	integral membrane protein	-5.1
STM0162	STM0162	inner membrane protein	-5.1
STM3960	*rhtB*	homoserine/homoserine lactone efflux protein	-5.1
STM0793	*bioA*	adenosylmethionine--8-amino-7-oxononanoate aminotransferase	-4.9
STM3959	*rhtC*	threonine efflux system	-4.9
STM1775	*hemK*	N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase	-4.9
STM3016	*araE*	L-arabinose/proton symport protein	-4.7
STM1320	*ydjN*	kinase/transporter-like protein	-4.7
STM0502	*ybbL*	ABC transporter ATP-binding protein	-4.7
STM2816	STM2816	glycoporin	-4.4
STM1242	*envE*	envelope protein	-4.4
STM1975	*fliL*	flagellar basal body-associated protein FliL	-4.4
STM1418	*ssaQ*	type III secretion system protein	-4.4

STM2754	STM2754	hexulose 6 phosphate synthase	-4.4
STM3383	*prmA*	50S ribosomal protein L11 methyltransferase	-4.4
STM1343	*nlpC*	lipoprotein	-4.4
STM0948	STM0948	hypothetical protein	-4.4
STM1429	*ydhB*	DNA-binding transcriptional regulator	-4.4
STM2823	STM2823	tRNA-Arg	-4.4
STM2848	*hycF*	formate hydrogenlyase complex iron-sulfur subunit	-4.4
STM1223	*potC*	spermidine/putrescine ABC transporter membrane protein	-4.4
STM1154	*yceE*	drug efflux system protein MdtG	-4.4
STM2022	*cbiN*	cobalt transport protein CbiN	-4.4
STM1694	*sapC*	peptide transport protein	-4.4
STM4271	STM4271	inner membrane protein	-4.4
STM3160	STM3160	inner membrane protein	-4.4
STM1808	STM1808	hypothetical protein	-4.4
STM4139	*coaA*	pantothenate kinase	-4.4
STM3277	STM3277	inner membrane protein	-4.4
STM2937	*ygbF*	hypothetical protein	-4.4
STM4141	STM4141	hypothetical protein	-4.4
STM1856	STM1856	hypothetical protein	-4.4
STM1858	STM1858	hypothetical protein	-4.4
STM1037	STM1037	minor tail protein	-4.4
STM4276	STM4276	hypothetical protein	-4.4
STM1219	*ycfW*	outer membrane-specific lipoprotein transporter subunit LolE	-4.4
STM4401	*ytfG*	reductase	-4.4
STM0381	STM0381	inner membrane protein	-4.4
STM2308	*yfbB*	acyl-CoA thioester hydrolase YfbB	-4.4
STM2118	*wza*	outer membrane polysaccharide export protein	-4.4
STM0699	STM0699	hypothetical protein	-4.4
STM0138	*yacG*	zinc-binding protein	-4.4
STM2508	STM2508	hypothetical protein	-4.4
STM2527	STM2527	polyferredoxin	-4.4
STM0033	STM0033	5'-nucleotidase	-4.4
STM0087	*folA*	dihydrofolate reductase	-4.4
STM1725	*trpC*	bifunctional indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase	-4.1
STM2846	*hycH*	hydrogenase 3 large subunit processing protein	-4.1
STM1678	STM1678	2'-hydroxyisoflavone reductase	-4.1
STM2641	*nadB*	L-aspartate oxidase	-4.0

STM0758	*ybgR*	zinc transporter ZitB	-4.0
STM3120	STM3120	transcriptional regulator	-4.0
STM2691	STM2691	ABC transporter	-4.0
STM1123	STM1123	hypothetical protein	-4.0
STM0746	*tolR*	colicin uptake protein TolR	-4.0
STM2018	*cobU*	adenosylcobinamide kinase/adenosylcobinamide-phosphate guanylyltransferase	-3.9
STM0230	*rnhB*	ribonuclease HII	-3.9
STM1026	STM1026	hypothetical protein	-3.9
STM0806	*moaE*	molybdopterin guanine dinucleotide biosynthesis protein MoaE	-3.9
STM3825	*torT*	TMAO reductase system periplasmic protein TorT	-3.8
STM3725	*coaD*	phosphopantetheine adenylyltransferase	-3.8
STM3795	*ilvN*	acetolactate synthase 1 regulatory subunit	-3.8
STM2833	*srlE*	glucitol/sorbitol-specific enzyme IIB component	-3.8
STM2102	*wzxC*	colanic acid exporter	-3.7
STM1649	STM1649	hypothetical protein	-3.7
STM0547	*fimH*	minor fimbrial subunit	-3.7
STM0613	STM0613	hydrogenase protein	-3.7
STM0582	*ybdJ*	inner membrane protein	-3.7
STM1966	*yedF*	hypothetical protein	-3.7
STM0179	*yadE*	xylanase/chitin deacetylase	-3.7
STM0544	*fimI*	fimbrial protein	-3.7
STM3910	*ppiC*	peptidyl-prolyl cis-trans isomerase C	-3.7
STM0983	*ycaI*	hypothetical protein	-3.7
STM3976.S	*yigW*	DNase TatD	-3.7
STM1279	*yeaM*	regulatory protein	-3.7
STM1064	*pqiB*	paraquat-inducible protein B	-3.7
STM2673	*rplS*	50S ribosomal protein L19	-3.7
STM2900	*invH*	needle complex outer membrane lipoprotein precursor	-3.5
STM1133	STM1133	dehydrogenase	-3.5
STM1065	*ymbA*	outer membrane protein	-3.5
STM1983	*dsrB*	hypothetical protein	-3.5
STM3727	*rpmG*	50S ribosomal protein L33	-3.4
STM3529	*gldA*	glycerol dehydrogenase	-3.4
STM2034	*cbiB*	cobalamin biosynthesis protein	-3.4
STM0109	*yabN*	transcriptional regulator SgrR	-3.3
STM1993	*yedJ*	hypothetical protein	-3.3

STM2126	STM2126	multidrug efflux system subunit MdtA	-3.3
STM1817	*rnd*	ribonuclease D	-3.3
STM2803	STM2803	regulatory protein	-3.2
STM0589	*fepE*	ferric enterobactin transport protein FepE	-3.2
STM3620	*yhjQ*	cell division protein	-3.2
STM0165	*speD*	S-adenosylmethionine decarboxylase	-3.2
STM3316	*yrbI*	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase	-3.2
STM3544	*yhhW*	hypothetical protein	-3.2
STM3848	*yidZ*	DNA-binding transcriptional regulator YidZ	-3.2
STM3364	*yhcP*	p-hydroxybenzoic acid efflux subunit AaeB	-3.2
STM2396	*pgtA*	activator	-3.1
STM2962	*gudT*	D-glucarate permease	-3.1
STM4284	*yjcO*	hypothetical protein	-3.1
STM3561	*livG*	leucine/isoleucine/valine transporter ATP-binding subunit	-3.1
STM2200	*lysP*	lysine transporter	-3.1
STM3692	*lldP*	L-lactate permease	-3.1
STM0292	STM0292	RHS-like protein	-3.1
STM0557	STM0557	inner membrane protein	-2.9
STM1698	STM1698	inner membrane protein	-2.9
STM1699	*ycjE*	hypothetical protein	-2.9
STM1843	STM1843	transporter	-2.9
STM2149	*stcD*	outer membrane lipoprotein	-2.9
STM0338	*stbC*	fimbrial usher	-2.9
STM3096	*yqgE*	hypothetical protein	-2.9
STM4113	*frwB*	PTS system fructose-like transporter subunit EIIB	-2.9
STM3066	*yggA*	arginine exporter protein	-2.9
STM0329	STM0329	isopropylmalate isomerase large subunit	-2.9
STM0084	STM0084	sulfatase	-2.9
STM2450	*amiA*	N-acetylmuramoyl-l-alanine amidase I	-2.9
STM1518	*marB*	hypothetical protein	-2.9
STM1745	*oppB*	oligopeptide transporter permease	-2.9
STM1526	*yneG*	hypothetical protein	-2.9
STM2617	STM2617	antiterminator-like protein	-2.9
STM3026	STM3026	outer membrane protein	-2.9
STM3024	*yohM*	nickel/cobalt efflux protein RcnA	-2.9
STM3143	*hybG*	hydrogenase 2 accessory protein HypG	-2.9
STM4551	STM4551	hypothetical protein	-2.9
STM1783.S	*pth*	peptidyl-tRNA hydrolase	-2.9

STM1579	*narW*	nitrate reductase 2 delta subunit	-2.9
STM0539	STM0539	inner membrane protein	-2.9
STM1156	*yceA*	hypothetical protein	-2.9
STM0523	*allB*	allantoinase	-2.9
STM3173	*plsC*	1-acyl-sn-glycerol-3-phosphate acyltransferase	-2.9
STM4082	*yiiQ*	hypothetical protein	-2.9
STM2863	*sitC*	permease	-2.9
STM0672	STM0672	inner membrane protein	-2.9
STM2535	*sseB*	enhanced serine sensitivity protein SseB	-2.9
STM2776	*iroE*	hydrolase	-2.9
STM1425	*ydhE*	multidrug efflux protein	-2.9
STM0884	*ulaA*	PTS system ascorbate-specific transporter subunit IIC	-2.9
STM4191	STM4191	hypothetical protein	-2.9
STM3823	*torC*	trimethylamine N-oxide reductase cytochrome c-like subunit	-2.9
STM0182	*panB*	3-methyl-2-oxobutanoate hydroxymethyltransferase	-2.9
STM0181	*panC*	pantoate--beta-alanine ligase	-2.9
STM0175	*stiC*	fimbrial usher	-2.9
STM2671	*yfiR*	hypothetical protein	-2.9
STM2263	*yojI*	multidrug transporter membrane protein/ATP-binding component	-2.9
STM1385	*ttrB*	tetrathionate reductase complex subunit B	-2.9
STM3942	STM3942	hypothetical protein	-2.9
STM3745	STM3745	hypothetical protein	-2.9
STM3780	*gatY*	fructose-1,6-bisphosphate aldolase	-2.9
STM4176	*purH*	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	-2.9
STM4589	*creC*	sensory histidine kinase CreC	-2.9
STM0651	STM0651	2-keto-3-deoxygluconate permease	-2.9
STM2030	*cbiT*	cobalt-precorrin-6Y C(15)-methyltransferase	-2.9
STM4473	*yjgM*	acetyltransferase	-2.9
STM1327	*ydiY*	outer membrane protein	-2.9
STM0657	*ybeU*	hypothetical protein	-2.9
STM4051	STM4051	outer membrane protein	-2.9
STM3693	*lldR*	DNA-binding transcriptional repressor LldR	-2.9
STM0707	*kdpF*	potassium-transporting ATPase subunit F	-2.9
STM1900	*ntpA*	dATP pyrophosphohydrolase	-2.9
STM0044	*yaaY*	hypothetical protein	-2.9
STM3444	*bfd*	bacterioferritin-associated ferredoxin	-2.9

STM1046	STM1046	tail assembly protein	-2.9
STM1040	STM1040	minor tail protein	-2.9
STM0780	STM0780	hypothetical protein	-2.9
STM2249	*ccmF*	cytochrome c-type biogenesis protein	-2.9
STM4382	*yjfR*	L-ascorbate 6-phosphate lactonase	-2.9
STM0035	STM0035	arylsulfatase	-2.9
STM2807	*nrdE*	ribonucleotide-diphosphate reductase subunit alpha	-2.9
STM1910	STM1910	penicillin-binding protein	-2.9
STM0704	*kdpC*	potassium-transporting ATPase subunit C	-2.9
STM1139	*csgG*	curli operon transcriptional regulator	-2.9
STM4196	STM4196	hypothetical protein	-2.9
STM4514.S	*yjiH*	inner membrane protein	-2.9
STM1032	STM1032	hypothetical protein	-2.9
STM2808	*nrdF*	ribonucleotide-diphosphate reductase subunit beta	-2.9
STM1031	STM1031	hypothetical protein	-2.8
STM1696	*sapF*	peptide transport protein	-2.8
STM0993	*mukE*	condesin subunit E	-2.7
STM3114	*speC*	ornithine decarboxylase	-2.7
STM3588	*yhiN*	hypothetical protein	-2.7
STM4076	*ydeZ*	sugar transport protein	-2.7
STM2499.S	*purM*	phosphoribosylaminoimidazole synthetase	-2.7
STM3872	*atpI*	F0F1 ATP synthase subunit I	-2.6
STM3242	*tdcD*	propionate/acetate kinase	-2.6
STM4538	STM4538	PTS permease	-2.6
STM1905	*yecO*	SAM-dependent methyltransferase	-2.6
STM0171	*yadF*	carbonic anhydrase	-2.6
STM3657	STM3657	outer membrane lipoprotein	-2.6
STM4266	*soxR*	redox-sensing transcriptional activator	-2.6
STM0419	*thiL*	thiamine monophosphate kinase	-2.6
STM1516	*ydeE*	MFS-type transporter YdeE	-2.6
STM3527	STM3527	hypothetical protein	-2.6
STM3971	*yigP*	inner membrane protein	-2.6
STM2434	STM2434	hypothetical protein	-2.6
STM3144	*hypA*	hydrogenase nickel incorporation protein HybF	-2.6
STM4481	*idnR*	L-idonate regulator	-2.6
STM1908	*yecM*	hypothetical protein	-2.6
STM1701	*yciW*	hypothetical protein	-2.6
STM2530	STM2530	anaerobic dimethylsulfoxide reductase	-2.6

STM1911	STM1911	hypothetical protein	-2.6
STM1083	*yccX*	acylphosphatase	-2.6
STM3628	*dppC*	dipeptide transporter	-2.6
STM3064	*iciA*	chromosome replication initiation inhibitor protein	-2.6
STM3623	*yhjT*	inner membrane protein	-2.6
STM1515	*ydeI*	hypothetical protein	-2.5
STM1392	*ssrA*	sensor kinase	-2.5
STM1754	*ychK*	hypothetical protein	-2.5
STM4286	*lpxO*	dioxygenase	-2.5
STM3585	*yhhJ*	ABC transport protein	-2.5
STM4550	*fhuF*	ferric hydroximate transport ferric iron reductase	-2.5
STM0111	*leuC*	isopropylmalate isomerase large subunit	-2.5
STM1496	STM1496	dimethylsulfoxide reductase	-2.5
STM1153	*msyB*	hypothetical protein	-2.5
STM0838	*ybiT*	ABC transporter ATPase	-2.5
STM0395	*sbcC*	exonuclease subunit SbcC	-2.5
STM0050	STM0050	nitrite reductase	-2.5
STM3868	*atpH*	F0F1 ATP synthase subunit delta	-2.5
STM0306	STM0306	adhesin/invasin protein PagN	-2.4
STM0363	STM0363	transcriptional regulator	-2.4
STM2498	*upp*	uracil phosphoribosyltransferase	-2.4
STM3555	*ugpE*	glycerol-3-phosphate transporter membrane protein	-2.4
STM2913	STM2913	permease	-2.4
STM0244	*rcsF*	outer membrane lipoprotein	-2.4
STM0431	*phnW*	2-aminoethylphosphonate--pyruvate transaminase	-2.4
STM1068	*lonH*	protease	-2.4
STM1868A	STM1868A	lytic enzyme	-2.4
STM0820	*rhlE*	ATP-dependent RNA helicase RhlE	-2.4
STM4574	STM4574	outer membrane protein	-2.4
STM0874	*mdaA*	nitroreductase A	-2.4
STM4316	STM4316	hypothetical protein	-2.4
STM2088	*rfbX*	O-antigen transferase	-2.4
STM1437	*ydhM*	transcriptional repressor	-2.4
STM0353	STM0353	cation transport ATPase	-2.4
STM1250	STM1250	hypothetical protein	-2.4
STM4066	STM4066	aminoimidazole riboside kinase	-2.4
STM1388	*orf70*	hypothetical protein	-2.4
STM4457	STM4457	transposase	-2.4

STM1767	*narL*	transcriptional regulator NarL	-2.4
STM2940	STM2940	hypothetical protein	-2.4
STM0510	*sfbA*	ABC transporter ATPase	-2.4
STM1598	*ydcR*	regulatory protein	-2.4
STM1593	*srfA*	virulence protein	-2.4
STM4347	*yjeP*	hypothetical protein	-2.4
STM3205	*uppP*	undecaprenyl pyrophosphate phosphatase	-2.4
STM4555	*leuQ*	tRNA-Leu	-2.4
STM0514	*ybbS*	DNA-binding transcriptional activator AllS	-2.4
STM1198	*pabC*	4-amino-4-deoxychorismate lyase	-2.4
STM0992	*mukF*	condesin subunit F	-2.4
STM2453	STM2453	hypothetical protein	-2.4
STM0159	STM0159	restriction endonuclease	-2.4
STM1193	*fabH*	3-oxoacyl-ACP synthase	-2.4
STM1082	STM1082	regulatory protein	-2.3
STM4588	*creB*	DNA-binding response regulator CreB	-2.3
STM2349	*yfcG*	glutathione S-transferase	-2.3
STM0145	*nadC*	quinolinate phosphoribosyltransferase	-2.3
STM0094	*djlA*	Dna-J like membrane chaperone protein	-2.3
STM0184	*pcnB*	poly(A) polymerase I	-2.3
STM2111	*wcaE*	glycosyl transferase family protein	-2.3
STM1823	*yoaH*	hypothetical protein	-2.3
STM2082	*rfbP*	undecaprenol-phosphate galactosephosphotransferase/O-antigen transferase	-2.3
STM1738	*yciI*	YciI-like protein	-2.3
STM3237	*yhaL*	hypothetical protein	-2.3
STM3315	*yrbH*	D-arabinose 5-phosphate isomerase	-2.3
STM0871	*ybjM*	inner membrane protein	-2.3
STM2930	*ispD*	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	-2.3
STM0311	*yafJ*	glutamine amidotransferase	-2.3
STM4398	*cycA*	D-alanine/D-serine/glycine permease	-2.3
STM2958	*barA*	hybrid sensory histidine kinase BarA	-2.3
STM3129	STM3129	NAD-dependent aldehyde dehydrogenase	-2.3
STM2674	*trmD*	tRNA (guanine-N(1)-)-methyltransferase	-2.2
STM2480	*narQ*	nitrate/nitrite sensor protein NarQ	-2.2
STM3928	*wecF*	common antigen polymerase	-2.2
STM3164	*yqhD*	alcohol dehydrogenase	-2.2
STM3528	STM3528	phosphate-binding protein	-2.2

STM1984	*yodD*	hypothetical protein	-2.2
STM0938	*ybjE*	inner membrane protein	-2.2
STM3503	*greB*	transcription elongation factor GreB	-2.2
STM3524	*glpG*	intramembrane serine protease GlpG	-2.2
STM0986	*ycaQ*	hypothetical protein	-2.2
STM3944	STM3944	inner membrane protein	-2.2
STM0727	STM0727	hypothetical protein	-2.2
STM4056.S	*yiiM*	hypothetical protein	-2.2
STM3847	*yidY*	multidrug efflux system protein MdtL	-2.2
STM0847	*ybiK*	L-asparaginase	-2.2
STM1151	*mdoH*	glucosyltransferase MdoH	-2.2
STM1011	STM1011	hypothetical protein	-2.2
STM0678	*leuW*	tRNA-Leu	-2.2
STM0535	*lpxH*	UDP-2,3-diacylglucosamine hydrolase	-2.2
STM3607	*yhjC*	transcriptional regulator	-2.2
STM3549	STM3549	inner membrane protein	-2.2
STM3989	*ileT*	tRNA-Ile	-2.2
STM3560	*livF*	leucine/isoleucine/valine transporter ATP-binding subunit	-2.2
STM2312	*elaA*	hypothetical protein	-2.2
STM0584	*entD*	phosphopantetheinyltransferase component of enterobactin synthase multienzyme complex	-2.2
STM0936	*hcr*	HCP oxidoreductase	-2.2
STM4079.S	*yneC*	autoinducer-2 (AI-2) modifying protein LsrG	-2.2
STM3956	*yigI*	hypothetical protein	-2.2
STM2254	*ccmA*	cytochrome c biogenesis protein CcmA	-2.2
STM4420	STM4420	inner membrane protein	-2.2
STM2783	*nixA*	nickel transporter	-2.2
STM1517	*ydeD*	O-acetylserine/cysteine export protein	-2.2
STM2796	*yqaE*	transporter	-2.2
STM3215	*yqjI*	transcriptional regulator	-2.2
STM4376	*yjfC*	glutathionylspermidine synthase	-2.2
STM1613	STM1613	PTS system enzymeIIB component	-2.2
STM1618	STM1618	transcriptional repressor of sgc operon	-2.2
STM1322	*yniC*	2-deoxyglucose-6-phosphatase	-2.2
STM3113	*nupG*	nucleoside transport	-2.2
STM2439	*yfeL*	membrane carboxypeptidase	-2.2
STM0359	STM0359	hypothetical protein	-2.2
STM0345	STM0345	inner membrane protein	-2.2
STM0343	STM0343	hypothetical protein	-2.2

STM4517	*yjiO*	transporter	-2.2
STM3098	STM3098	transcriptional regulator	-2.2
STM4523	*yjiW*	endoribonuclease SymE	-2.2
STM2252	*ccmC*	heme exporter protein	-2.2
STM2128	*yegO*	multidrug efflux system subunit MdtC	-2.2
STM4477	*pepA*	leucyl aminopeptidase	-2.2
STM4353	*glyX*	tRNA-Gly	-2.2
STM4423	STM4423	DNA-binding protein	-2.2
STM4451	*nrdG*	anaerobic ribonucleotide reductase-activating protein	-2.2
STM2854	*hypA*	hydrogenase nickel incorporation protein	-2.2
STM1482	*ydgF*	multidrug efflux system protein MdtJ	-2.2
STM4472	*ytgA*	inner membrane protein	-2.2
STM1390	*orf242*	regulatory protein	-2.2
STM2294	*yfaZ*	inner membrane protein	-2.2
STM3324	*ptsO*	phosphohistidinoprotein-hexose phosphotransferase component of N-regulated PTS system (Npr)	-2.2
STM2008	STM2008	hypothetical protein	-2.2
STM3374.1n	STM3374.1n	hypothetical protein	-2.2
STM2262	*eco*	ecotin	-2.2
STM1472	STM1472	hypothetical protein	-2.2
STM1826	*sdaA*	L-serine deaminase I/L-threonine deaminase I	-2.1
STM1814	*minC*	septum formation inhibitor	-2.1
STM2944	*ygcB*	helicase	-2.1
STM1307	*astE*	succinylglutamate desuccinylase	-2.1
STM1049	STM1049	tail fiber protein	-2.1
STM3112	*mltC*	murein transglycosylase C	-2.1
STM3593	*yhiQ*	methyltransferase	-2.1
STM3334	STM3334	cytosine deaminase	-2.1
STM2364	*dedD*	hypothetical protein	-2.1
STM3498	*hslO*	Hsp33-like chaperonin	-2.1
STM4294	*yjdE*	arginine:agmatin antiporter	-2.1
STM1691	*pspF*	phage shock protein operon transcriptional activator	-2.1
STM2685	*smpA*	hypothetical protein	-2.1
STM3794	STM3794	regulatory protein	-2.1
STM1617	STM1617	epimerase	-2.1
STM3955	*rarD*	chloramphenicol resistance	-2.1
STM3619	*bcsA*	cellulose synthase catalytic subunit	-2.1
STM2858	*hypE*	hydrogenase formation protein	-2.1

STM2282	*glpQ*	glycerophosphodiester phosphodiesterase	-2.1
STM0212	STM0212	inner membrane protein	-2.1
STM2487	*purC*	phosphoribosylaminoimidazole-succinocarboxamide synthase	-2.1
STM0760	*aroG*	phospho-2-dehydro-3-deoxyheptonate aldolase	-2.1
STM1355	*ydiP*	transcriptional regulator	-2.0
STM0333	STM0333	transcriptional regulator	-2.0
STM3900	*ilvL*	ilvG operon leader peptide	-2.0
STM0801	*ybhK*	hypothetical protein	-2.0
STM3754	STM3754	hypothetical protein	-2.0
STM4223	*yjbF*	outer membrane lipoprotein	-2.0
STM0086	*kefC*	glutathione-regulated potassium-efflux system protein KefC	-2.0
STM2584	*gogB*	hypothetical protein	-2.0
STM2157	*yehS*	hypothetical protein	-2.0
STM1582	*nhoA*	arylamine N-acetyltransferase	-2.0
STM1931	*araH*	intracellular protease/amidase	-2.0
STM1797	*ymgE*	transglycosylase-associated protein	-2.0
STM3946	*yifL*	outer membrane lipoprotein	-2.0
STM0459	*ybaO*	transcriptional regulator	-2.0
STM0424	*xseB*	exodeoxyribonuclease VII small subunit	-2.0
STM1450	*pdxY*	pyridoxamine kinase	-2.0
STM2016	*cobT*	nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase	-2.0
STM2485	*ypfI*	acetyltransferase	-2.0
STM3799	STM3799	hypothetical protein	-2.0
STM2596	STM2596	minor tail-like protein	-2.0
STM1489	*bioD*	dithiobiotin synthetase	-2.0
Up-regulated genes			
Locus	**Gene name**	**Function**	**Fold change**
STM3933	*leuT*	tRNA-Leu	12.3
STM1399	*sscA*	secretion system chaperone	12.3
STM3890	*gltU*	tRNA-Glu	10.5
STM3238	*yhaN*	inner membrane protein	9.6
STM3350	STM3350	inner membrane protein	8.9
STM3829	*dgoK*	2-oxo-3-deoxygalactonate kinase	8.2
STM1280	*yeaL*	inner membrane protein	8.2
STM0642	*ybeB*	hypothetical protein	6.8
STM4516	*yjiN*	inner membrane protein	6.8
STM2782	*mig-14*	transcriptional activator	6.8

STM0173	*yadH*	transporter	6.8
STM2842	*hypF*	hydrogenase maturation protein	6.8
STM0163	*pdxA*	4-hydroxythreonine-4-phosphate dehydrogenase	6.5
STM0135	*yacA*	SecA regulator SecM	6.2
STM2448	*yfeZ*	inner membrane protein	5.5
STM3932	*hisR*	tRNA-His	5.5
STM0387	*yaiI*	hypothetical protein	5.5
STM4549	STM4549	hypothetical protein	5.5
STM3133	STM3133	amidohydrolase	5.5
STM4593	*sthB*	fimbrial usher protein	5.5
STM0268	STM0268	hypothetical protein	5.5
STM1050	STM1050	tail fiber assembly like-protein	5.5
STM2288	STM2288	hypothetical protein	5.5
STM0183	*folK*	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	5.5
STM0006	*yaaJ*	alanine/glycine transport protein	5.5
STM0001	*thrL*	thr operon leader peptide	4.8
STM1352	*ydiS*	hypothetical protein	4.8
STM1269	STM1269	chorismate mutase	4.8
STM3645	*yiaD*	outer membrane lipoprotein	4.8
STM2836	*gutM*	DNA-binding transcriptional activator GutM	4.8
STM4298	*melA*	alpha-galactosidase	4.8
STM0511	*sfbB*	ABC transporter ATPase	4.6
STM2628	STM2628	regulatory protein	4.5
STM0991	*smtA*	metallothionein SmtA	4.4
STM3169	STM3169	periplasmic dicarboxylate-binding protein	4.4
STM1762	*narJ*	nitrate reductase 1 delta subunit	4.4
STM0095	*rluA*	23S rRNA/tRNA pseudouridine synthase A	4.4
STM4571	STM4571	outer membrane protein	4.1
STM2139	STM2139	inner membrane protein	4.1
STM1874	STM1874	inner membrane protein	4.1
STM1877	STM1877	amidohydrolase	4.1
STM2612	STM2612	morphogenesis-like protein	4.1
STM2503	STM2503	diguanylate cyclase	4.1
STM2747	STM2747	hypothetical protein	4.1
STM3387	*yhdU*	hypothetical protein	4.1
STM0907	STM0907	chitinase	4.1
STM0263	*rnhA*	ribonuclease H	4.1

STM1523	*yneJ*	transcriptional regulator	4.1
STM4278.S	*nrfB*	cytochrome c nitrite reductase pentaheme subunit	4.1
STM0646	*holA*	DNA polymerase III subunit delta	4.1
STM0794	*bioB*	biotin synthetase	4.1
STM3275.S	*yhbV*	protease	4.1
STM1442	*ydhJ*	multidrug resistance efflux pump	4.1
STM0859	STM0859	transcriptional regulator	4.1
STM3792	STM3792	L-fucose permease	4.1
STM4399	*ytfE*	iron-sulfur cluster repair di-iron protein	4.1
STM1974	*fliK*	flagellar hook-length control protein	3.9
STM2306	*menC*	O-succinylbenzoate synthase	3.8
STM4469	*argI*	ornithine carbamoyltransferase subunit I	3.8
STM0821	*dinG*	ATP-dependent DNA helicase DinG	3.7
STM1397	*sseA*	secretion system chaperone protein	3.7
STM2202	*yeiH*	inner membrane protein	3.7
STM3986	*trkH*	potassium transporter	3.7
STM4287.S	*phnO*	aminoalkylphosphonic acid N-acetyltransferase	3.7
STM4591	*sthE*	major fimbrial subunit	3.4
STM4554	*leuP*	tRNA-Leu	3.4
STM0185	*yadB*	glutamyl-Q tRNA(Asp) synthetase	3.4
STM1973	*fliJ*	flagellar biosynthesis chaperone	3.4
STM2938	STM2938	hypothetical protein	3.4
STM4308	STM4308	anaerobic dehydrogenase component	3.4
STM4435	STM4435	hypothetical protein	3.4
STM1522	*ydeA*	sugar efflux transporter	3.4
STM1794	STM1794	membrane protein	3.4
STM4319	*phoN*	non-specific acid phosphatase	3.4
STM1549	STM1549	translation initiation inhibitor	3.4
STM3178	*ygiY*	sensor protein QseC	3.4
STM3168	*ygiR*	hypothetical protein	3.4
STM1821	*yoaA*	DNA helicase	3.4
STM1913	*flhA*	flagellar biosynthesis protein FlhA	3.4
STM0229	*lpxB*	lipid-A-disaccharide synthase	3.4
STM4511	*yjiE*	DNA-binding transcriptional regulator	3.4
STM3441	*rpsJ*	30S ribosomal protein S10	3.4
STM0023	*bcfC*	fimbrial usher	3.4
STM2394	*argW*	tRNA-Arg	3.4
STM4263	*yjcB*	inner membrane protein	3.4
STM4186	STM4186	hypothetical protein	3.4

STM3828	*dgoA*	galactonate dehydratase	3.4
STM2794	*ygaE*	DNA-binding transcriptional regulator CsiR	3.4
STM2496	*yfgE*	DNA replication initiation factor	3.4
STM1994	STM1994	inner membrane protein	3.4
STM0681	*nagD*	UMP phosphatase	3.3
STM0881	*ybjO*	inner membrane protein	3.2
STM0254	*aspU*	tRNA-Asp	3.2
STM2945	*sopD*	secreted effector protein	3.1
STM0877	*potF*	putrescine ABC transporter periplasmic-binding protein	3.1
STM1134	*serX*	tRNA-Ser	3.1
STM4090	*menA*	1,4-dihydroxy-2-naphthoate octaprenyltransferase	3.1
STM3346	*yhcM*	ATPase	3.1
STM1228	STM1228	hypothetical protein	3.1
STM3612	*kdgK*	ketodeoxygluconokinase	3.1
STM0041	STM0041	glycosyl hydrolase	3.1
STM2199	*cirA*	colicin I receptor	3.1
STM2654	*kgtP*	alpha-ketoglutarate transporter	3.1
STM1170	*mviN*	virulence protein	3.0
STM4508	*trpS2*	tryptophanyl-tRNA synthetase II	3.0
STM3118	STM3118	acetyl-CoA hydrolase	3.0
STM3926	*wzxE*	O-antigen translocase	3.0
STM3644	*bisC*	biotin sulfoxide reductase	3.0
STM1202	*ycfH*	metallodependent hydrolase	3.0
STM3475	*nirD*	nitrite reductase small subunit	3.0
STM2026	*cbiJ*	cobalt-precorrin-6x reductase	3.0
STM3765	*yicL*	permease	3.0
STM2570	STM2570	phosphotransferase system IIB component	3.0
STM4252	STM4252	inner membrane protein	3.0
STM2129	*yegB*	multidrug efflux system protein MdtE	2.9
STM1693	*sapB*	peptide transport protein	2.9
STM1491	*osmV*	proline/glycine betaine transport systems	2.9
STM1499	STM1499	dimethyl sulfoxide reductase subunit A	2.8
STM0201	STM0201	outer membrane protein	2.7
STM2309	*menD*	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase	2.7
STM1687	*pspD*	peripheral inner membrane phage-shock protein	2.7
STM2021	*cbiQ*	vitamin B12 biosynthetic protein	2.7
STM1024	STM1024	hypothetical protein	2.7
STM1042	STM1042	minor tail protein	2.7

STM2679	*yfjD*	hypothetical protein	2.7
STM1084	*yccK*	sulfur transfer protein TusE	2.7
STM4031	STM4031	hypothetical protein	2.7
STM0626	*dpiA*	two-component response regulator DpiA	2.7
STM0621	*citF*	citrate lyase alpha chain/citrate-ACP transferase	2.7
STM1105	*hpaH*	4-hydroxyphenylacetate catabolism	2.7
STM0580	STM0580	regulatory protein	2.7
STM4112	*frwC*	fructose-like permease EIIC subunit 2	2.7
STM0564	STM0564	pyridine nucleotide-disulfide oxidoreductase	2.7
STM2143	*yegU*	glycohydrolase	2.7
STM1201	*holB*	DNA polymerase III subunit delta'	2.7
STM3574	*yhhM*	inner membrane protein	2.7
STM4230	*malK*	maltose/maltodextrin transporter ATP-binding protein	2.7
STM0396	*sbcD*	exonuclease subunit SbcD	2.7
STM3102	*yggU*	hypothetical protein	2.7
STM2223	*yejH*	ATP-dependent helicase	2.7
STM0303	*ybeJ*	xylanase/chitin deacetylase	2.7
STM3222	*ygjQ*	integral membrane protein	2.7
STM0331	STM0331	fumarylacetoacetate hydrolase	2.7
STM3284	*truB*	tRNA pseudouridine synthase B	2.7
STM2031	*cbiE*	cobalt-precorrin-6Y C(5)-methyltransferase	2.7
STM2887	*spaS*	surface presentation of antigens protein SpaS	2.7
STM1410	STM1410	hypothetical protein	2.7
STM1218	*lolD*	lipoprotein transporter ATP-binding subunit	2.7
STM3332	*yhcG*	hypothetical protein	2.7
STM2084	*rfbM*	mannose-1-phosphate guanylyltransferase	2.7
STM3949	*xerC*	site-specific tyrosine recombinase XerC	2.6
STM4032	STM4032	acetyl esterase	2.6
STM1709	*yciS*	inner membrane protein	2.6
STM1586	STM1586	hypothetical protein	2.6
STM3372	*mreD*	rod shape-determining protein MreD	2.6
STM3712	*rfaC*	ADP-heptose--LPS heptosyltransferase 1	2.5
STM2505	STM2505	inner membrane protein	2.5
STM4033	STM4033	regulatory protein	2.5
STM3608	*yhjD*	tRNA-processing ribonuclease	2.5
STM0426	*phnV*	2-aminoethylphosphonate transporter	2.5
STM4121	*argC*	N-acetyl-gamma-glutamyl-phosphate reductase	2.5
STM1379	*orf48*	amino acid permease	2.5
STM0968	*ycaD*	MFS family transporter protein	2.5

STM0846	*moeA*	molybdopterin biosynthesis protein MoeA	2.5
STM3681	STM3681	transcriptional regulator	2.5
STM2597	STM2597	major tail-like protein	2.4
STM1498	STM1498	dimethyl sulfoxide reductase	2.4
STM3952	*corA*	magnesium/nickel/cobalt transporter CorA	2.4
STM4228	*malF*	maltose transporter membrane protein	2.4
STM3764	*mgtC*	Mg2+ transport protein	2.4
STM2917	*ygbK*	tRNA synthase	2.4
STM2619	STM2619	hypothetical protein	2.4
STM0878	*potG*	putrescine ABC transporter ATP-binding protein	2.4
STM0382	STM0382	permease	2.4
STM3288	*yhbC*	hypothetical protein	2.4
STM2531	*pbpC*	penicillin-binding protein 1C	2.4
STM0410	STM0410	regulatory protein	2.4
STM1547	STM1547	transcriptional regulator	2.4
STM0802	*moaA*	molybdenum cofactor biosynthesis protein A	2.4
STM0406	*yajC*	preprotein translocase subunit YajC	2.4
STM3796A.S	STM3796A.S	integral membrane protein	2.4
STM0619	*citG*	triphosphoribosyl-dephospho-CoA synthase	2.4
STM4171	*yjaH*	inner membrane protein	2.4
STM3001	*thyA*	thymidylate synthase	2.4
STM2591	STM2591	tail assembly protein K-like	2.4
STM4107	*yijF*	hypothetical protein	2.4
STM1321	*ydjM*	hypothetical protein	2.4
STM3661	*xylA*	xylose isomerase	2.4
STM1706	*yciH*	translation initiation factor Sui1	2.4
STM1771	*chaA*	calcium/sodium:proton antiporter	2.4
STM3055	*gcvT*	glycine cleavage system aminomethyltransferase T	2.4
STM2768	STM2768	transposase	2.4
STM0618	*citT*	citrate/succinate transport antiport protein	2.4
STM1857	STM1857	acetyltransferase	2.4
STM2339	*yfcC*	hypothetical protein	2.4
STM2353	*hisQ*	histidine/lysine/arginine/ornithine transport protein	2.4
STM3241	*tdcE*	pyruvate formate-lyase 4/2-ketobutyrate formate-lyase	2.3
STM3523	*glpR*	DNA-binding transcriptional repressor GlpR	2.3
STM4229	*malE*	maltose ABC transporter periplasmic protein	2.3
STM2607	STM2607	head-to-tail joining-like protein	2.3
STM3199	*yqiK*	hypothetical protein	2.3

STM4485	*idnK*	D-gluconate kinase	2.3
STM4129	*trmA*	tRNA (uracil-5-)-methyltransferase	2.3
STM3089	*yqgD*	inner membrane protein	2.3
STM1893	*znuB*	high-affinity zinc transporter membrane protein	2.3
STM1806	*nhaB*	sodium/proton antiporter	2.3
STM2630	STM2630	hypothetical protein	2.3
STM4542	*yjjA*	hypothetical protein	2.3
STM0221	*uppS*	undecaprenyl pyrophosphate synthase	2.2
STM0950	STM0950	SlsA	2.2
STM1739	*cls*	cardiolipin synthetase	2.2
STM4317	STM4317	hypothetical protein	2.2
STM1605	*ydcN*	repressor	2.2
STM3373	*mreC*	rod shape-determining protein MreC	2.2
STM3467	*yhfK*	inner membrane protein	2.2
STM0081	STM0081	hypothetical protein	2.2
STM3341	*sspB*	ClpXP protease specificity-enhancing factor	2.2
STM3870	*atpE*	F0F1 ATP synthase subunit C	2.2
STM0260	*mltD*	membrane-bound lytic murein transglycosylase D	2.2
STM1937	*tyrP*	tyrosine-specific transport protein	2.2
STM0116	*ilvI*	acetolactate synthase 3 catalytic subunit	2.2
STM2506	STM2506	inner membrane protein	2.2
STM1136	*ycdX*	hydrolase	2.2
STM2663	*yfiO*	outer membrane protein assembly complex subunit YfiO	2.2
STM1059	*ycbW*	hypothetical protein	2.2
STM1731	STM1731	catalase	2.2
STM1735	*yciB*	intracellular septation protein A	2.2
STM1188	STM1188	inner membrane lipoprotein	2.1
STM4262	STM4262	ABC-type bacteriocin/lantibiotic exporter	2.1
STM4346	*yjeO*	inner membrane protein	2.1
STM1820	*yeaZ*	molecular chaperone	2.1
STM0285	STM0285	inner membrane protein	2.1
STM0287	STM0287	hypothetical protein	2.1
STM2275	STM2275	regulatory protein	2.1
STM3124	STM3124	response regulator	2.1
STM3126	STM3126	amino acid transporter	2.1
STM1835	*rrmA*	23S rRNA methyltransferase A	2.1
STM4320	STM4320	regulatory protein	2.1
STM1761	*narI*	nitrate reductase 1 gamma subunit	2.1

STM1773	*ychA*	transcriptional regulator	2.1
STM4365	*yjeT*	inner membrane protein	2.1
STM1898	*ruvC*	Holliday junction resolvase	2.1
STM2973	*fucO*	L-1,2-propanediol oxidoreductase	2.1
STM4335	*ecnA*	entericidin A precursor	2.1
STM2989	*metZ*	tRNA-Met	2.1
STM3079.S	STM3079.S	hydrolase/acyltransferase	2.1
STM4304	*dcuS*	sensory histidine kinase DcuS	2.1
STM2301	*arnT*	4-amino-4-deoxy-L-arabinose transferase	2.1
STM1789	STM1789	hydrogenase 1 maturation protease	2.1
STM3037	*glyU*	tRNA-Gly	2.1
STM1733	STM1733	ferredoxin	2.1
STM4143	*tyrU*	tRNA-Tyr	2.1
STM1401	*sseD*	translocation machinery component	2.1
STM3357	STM3357	regulatory protein	2.1
STM0481	*priC*	primosomal replication protein N''	2.1
STM4069	STM4069	hypothetical protein	2.1
STM0674	*glnV*	tRNA-Gln	2.1
STM3374	*mreB*	rod shape-determining protein MreB	2.1
STM0503	*ybbM*	transporter	2.1
STM3994	*mobA*	molybdopterin-guanine dinucleotide biosynthesis protein MobA	2.1
STM3667	*yiaJ*	transcriptional repressor	2.1
STM3714	*rfaK*	hexose transferase	2.1
STM0177	*stiA*	fimbrial subunit	2.1
STM0568	*pheP*	phenylalanine transporter	2.1
STM1089	STM1089	inner membrane protein	2.1
STM1093	STM1093	hypothetical protein	2.1
STM0581	STM0581	regulatory protein	2.1
STM2586	STM2586	phage tail assembly-like protein	2.1
STM0598	*entA*	2,3-dihydroxybenzoate-2,3-dehydrogenase	2.1
STM3531	STM3531	dihydroxyacid dehydratase	2.1
STM3182	*yqiA*	esterase YqiA	2.1
STM3451	*yheN*	sulfur transfer complex subunit TusD	2.1
STM4178	*gltV*	tRNA-Glu	2.1
STM4449	STM4449	bifunctional antitoxin/transcriptional repressor RelB	2.1
STM0866	*mdfA*	multidrug translocase	2.1
STM0835	STM0835	manganese transport regulator MntR	2.1
STM4594	*sthA*	fimbrial chaperone	2.1

STM3821	*torD*	chaperone protein TorD	2.1
STM3805	*yidH*	inner membrane protein	2.1
STM2824	STM2824	tRNA-Arg	2.1
STM2511	*guaB*	inosine 5'-monophosphate dehydrogenase	2.1
STM2099	*wcaM*	colanic acid biosynthesis protein	2.1
STM4247	*alr*	alanine racemase	2.1
STM2122	*udk*	uridine kinase	2.1
STM2125	*yegD*	chaperone	2.1
STM1488	*mlc*	pts operon transcriptional repressor	2.1
STM3732	*slmA*	nucleoid occlusion protein	2.1
STM3461	STM3461	hypothetical protein	2.0
STM2097	*rfbB*	dTDP-glucose-4,6-dehydratase	2.0
STM2145	*yegW*	regulatory protein	2.0

Appendix 4. List of the 734 differentially expressed proteins (*p*-value < 0.05) identified by Scaffold Q+.

The first 175 proteins have a cluster number associated and are the final selection yielded by the PCA and hierarchical clustering analysis.

Identified proteins	Permutation test (p-value)	log_2 (fold change)					Cluster
		After Dryer	a_w 0.11	a_w 0.11 thermal	a_w 1.0	a_w 1.0 thermal	
Stringent starvation protein A OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sspA PE=3 SV=1	< 0.0001	-0.480	-0.491	-0.561	-0.103	-0.111	1
Putative membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yggB PE=4 SV=1	< 0.0001	-0.399	-0.256	-0.358	0.094	-0.195	1
Methyl-accepting chemotaxis protein I OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tsr PE=4 SV=1	< 0.0001	-0.370	-0.345	-0.327	-0.085	-0.201	1
Copper-exporting P-type ATPase A OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=copA PE=1 SV=3	< 0.0001	-0.343	-0.407	-0.297	-0.092	-0.257	1
Shikimate kinase 1 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aroK PE=3 SV=1	< 0.0001	-0.370	-0.374	-0.386	-0.245	-0.298	1
Glycoprotein/polysaccharide metabolism OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ybaY PE=4 SV=1	< 0.0001	-0.587	-0.536	-0.563	-0.208	-0.233	1
PanD maturation factor OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=panM PE=1 SV=1	0.00015	-0.501	-0.489	-0.423	-0.148	-0.109	1
Outer-membrane lipoprotein LolB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=lolB PE=3 SV=1	0.00016	-0.447	-0.416	-0.424	-0.069	-0.173	1
Acridine efflux pump OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=acrB PE=3 SV=1	0.00025	-0.373	-0.418	-0.466	-0.100	-0.275	1
Putative phosphatase in N-acetylglucosamine metabolism OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nagD PE=4 SV=1	0.00038	-0.328	-0.426	-0.348	-0.216	-0.240	1
Putative translation initiation inhibitor OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yoaB PE=1 SV=1	0.00048	-0.530	-0.439	-0.509	-0.069	-0.015	1
Putative periplasmic immunogenic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yggE PE=4 SV=1	0.001	-0.512	-0.477	-0.409	-0.115	-0.036	1

3'-5' ssDNA/RNA exonuclease TatD OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tatD PE=3 SV=1	0.001	-0.567	-0.481	-0.288	-0.161	-0.180	1
NAD-dependent fermentative D-lactate dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ldhA PE=3 SV=1	0.002	-0.364	-0.355	-0.382	-0.252	-0.278	1
D-alanine--D-alanine ligase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ddl_2 PE=3 SV=1	0.0022	-0.364	-0.313	-0.445	-0.073	-0.235	1
Thioredoxin 1 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=trxA PE=3 SV=2	0.0022	-0.483	-0.492	-0.459	-0.165	-0.035	1
Outer-membrane lipoprotein carrier protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=lolA PE=3 SV=1	0.003	-0.484	-0.410	-0.233	-0.192	-0.131	1
Peptidyl-prolyl cis-trans isomerase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fklB PE=3 SV=1	0.006	-0.346	-0.343	-0.404	-0.239	-0.287	1
Protein CreA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=creA PE=4 SV=1	0.008	-0.534	-0.513	-0.533	-0.181	-0.113	1
Bifunctional uridylyltransferase/uridylyl-removing enzyme OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glnD PE=3 SV=1	0.011	-0.732	-0.437	-0.387	-0.053	-0.125	1
6-phosphogluconate dehydratase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=edd PE=3 SV=1	0.04	-0.266	-0.369	-0.387	-0.227	-0.216	1
Outer membrane protein assembly factor BamB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfgL PE=3 SV=1	< 0.0001	-0.433	-0.350	-0.473	0.107	0.099	2
Alpha-helical coiled coil protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tlpA PE=4 SV=1	< 0.0001	-0.421	-0.445	-0.382	-0.080	-0.065	2
3-dehydroquinate dehydratase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aroD PE=3 SV=1	< 0.0001	-0.339	-0.311	-0.353	-0.046	-0.035	2
Keto-hydroxyglutarate-aldolase/keto-deoxy-phosphogluconate aldolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=eda PE=4 SV=1	< 0.0001	-0.318	-0.382	-0.422	-0.177	-0.137	2
Glutathione oxidoreductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=gor PE=3 SV=1	< 0.0001	-0.367	-0.358	-0.307	0.019	-0.022	2
N-ethylmaleimide reductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nemA PE=4 SV=1	< 0.0001	-0.391	-0.364	-0.372	-0.053	-0.059	2
Thymidine kinase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tdk_2 PE=3 SV=1	0.00017	-0.391	-0.332	-0.442	0.061	-0.007	2

Small heat shock protein IbpB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ibpB PE=3 SV=1	0.0006	-0.350	-0.268	-0.441	-0.002	-0.044	2
Agmatinase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=speB PE=3 SV=1	0.00077	-0.322	-0.483	-0.434	0.100	0.023	2
Cell division protein ZapA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=zapA PE=3 SV=1	0.00089	-0.327	-0.357	-0.295	0.026	0.021	2
Periplasmic glycerophosphodiester phosphodiesterase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=glpQ PE=4 SV=1	0.0038	-0.579	-0.492	-0.515	0.089	0.024	2
Maltose ABC transporter periplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=malE PE=4 SV=1	0.004	-0.396	-0.369	-0.447	-0.123	-0.087	2
L-asparaginase II OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ansB PE=3 SV=1	0.011	-0.465	-0.294	-0.463	0.035	-0.001	2
Conjugative transfer: surface exclusion OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=traT PE=4 SV=1	0.017	-0.375	-0.381	-0.363	-0.071	-0.012	2
Ribulose-phosphate 3-epimerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpe PE=3 SV=1	0.018	-0.301	-0.279	-0.374	0.013	0.010	2
Glucose-specific PTS system component OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=crr PE=4 SV=1	0.03	-0.387	-0.416	-0.427	-0.160	-0.105	2
Deoxyuridine 5'-triphosphate nucleotidohydrolase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dut PE=3 SV=1	0.035	-0.596	-0.326	-0.565	0.034	0.114	2
Putative ABC transporter periplasmic binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_1515 PE=4 SV=1	0.039	-0.424	-0.371	-0.318	-0.100	-0.021	2
Methyl-accepting chemotaxis protein III OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=trg PE=4 SV=2	< 0.0001	-0.339	-0.225	-0.217	0.132	-0.014	3
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yedD PE=4 SV=1	< 0.0001	-0.281	-0.260	-0.250	0.008	0.096	3
Uracil phosphoribosyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=upp PE=3 SV=1	< 0.0001	-0.254	-0.237	-0.264	-0.034	0.038	3
D-aminoacyl-tRNA deacylase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yihZ PE=3 SV=1	0.001	-0.329	-0.267	-0.306	-0.099	0.040	3
DNA replication terminus site-binding protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tus	0.001	-0.289	-0.202	-0.318	0.052	0.005	3

PE=3 SV=2							
1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=hisA PE=1 SV=2	0.0014	-0.392	-0.232	-0.160	0.004	0.006	3
Thiol:disulfide interchange protein DsbC OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=dsbC PE=4 SV=1	0.002	-0.288	-0.317	-0.206	-0.085	0.055	3
Peptidase E OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pepE PE=3 SV=1	0.005	-0.282	-0.254	-0.226	0.007	0.074	3
Serine--tRNA ligase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=serS PE=3 SV=1	0.01	-0.289	-0.203	-0.220	-0.004	0.012	3
Inosine-5'-monophosphate dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=guaB PE=3 SV=1	0.011	-0.250	-0.302	-0.251	-0.099	-0.059	3
Cytidine deaminase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cdd PE=3 SV=1	0.014	-0.334	-0.299	-0.294	-0.048	0.080	3
Deoxyribose-phosphate aldolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=deoC PE=3 SV=1	0.016	-0.257	-0.265	-0.243	-0.081	-0.065	3
Beta-hexosaminidase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nagZ PE=1 SV=1	0.019	-0.332	-0.095	-0.307	0.041	-0.034	3
Phosphate-binding protein PstS OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pstS PE=3 SV=1	0.022	-0.271	-0.183	-0.187	-0.083	0.012	3
Bifunctional 2',3'-cyclic nucleotide 2'-phosphodiesterase/3'-nucleotidase periplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cpdB PE=3 SV=1	0.024	-0.266	-0.301	-0.252	-0.016	-0.035	3
Putative oxidoreductase component OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ycdY PE=4 SV=1	0.032	-0.329	-0.185	-0.302	0.047	0.008	3
Protease HtpX OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=htpX PE=3 SV=1	0.048	-0.274	-0.256	-0.236	0.140	-0.018	3
Peptidase B OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pepB PE=3 SV=1	< 0.0001	-0.174	-0.218	-0.152	0.159	0.198	4
Outer membrane protein assembly factor BamA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yaeT PE=3 SV=1	< 0.0001	-0.135	-0.133	-0.136	0.107	0.129	4
Putative periplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yraP	< 0.0001	-0.078	-0.103	-0.079	0.117	0.209	4

PE=4 SV=1							
Glutamyl-tRNA synthetase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=gltX PE=3 SV=1	< 0.0001	-0.111	-0.136	-0.162	0.215	0.201	4
Universal stress protein G OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=uspG PE=3 SV=1	< 0.0001	-0.223	-0.238	-0.148	0.184	0.196	4
ATP synthase subunit beta OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=atpD PE=3 SV=1	< 0.0001	-0.125	-0.135	-0.146	0.050	0.101	4
Spermidine/putrescine-binding periplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=potD PE=3 SV=1	< 0.0001	-0.175	-0.240	-0.154	0.147	0.215	4
Outer membrane protein assembly factor BamD OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yfiO PE=3 SV=1	< 0.0001	-0.211	-0.250	-0.217	0.118	0.152	4
Bifunctional purine biosynthesis protein PurH OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=purH PE=3 SV=1	< 0.0001	-0.150	-0.134	-0.088	0.173	0.232	4
Molybdenum cofactor biosynthesis protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=mogA PE=4 SV=1	< 0.0001	-0.211	-0.330	-0.305	0.102	0.129	4
Flagellar hook protein FlgE OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=flgE PE=1 SV=2	< 0.0001	-0.167	-0.125	-0.171	0.232	0.259	4
Protein translocase subunit SecD OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=secD PE=3 SV=1	< 0.0001	-0.150	-0.201	-0.128	0.171	0.010	4
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_2476 PE=4 SV=1	< 0.0001	-0.189	-0.238	-0.259	0.248	0.204	4
Phase 2 flagellin OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fljB PE=3 SV=2	< 0.0001	-0.150	-0.224	-0.286	0.128	0.209	4
Methylglyoxal synthase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=mgsA PE=3 SV=1	0.00012	-0.113	-0.220	-0.141	0.134	0.146	4
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yicH PE=4 SV=1	0.00018	-0.354	-0.182	-0.111	0.342	0.071	4
Putative ABC transporter ATP-binding protein YhbG OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yhbG PE=4 SV=1	0.00029	-0.107	-0.105	-0.189	0.152	0.092	4
Penicillin-binding protein activator LpoA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yraM PE=3 SV=1	0.00039	-0.077	-0.136	-0.150	0.110	0.224	4
Ubiquinol oxidase subunit 2 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cyoA	0.00039	-0.169	-0.177	-0.220	0.188	0.064	4

PE=3 SV=1							
Protein-export membrane protein SecF OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=secF PE=3 SV=1	0.00071	-0.192	-0.156	-0.210	0.237	0.098	4
Sensor protein BasS OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=basS PE=1 SV=1	0.00089	-0.221	-0.113	-0.183	0.143	0.055	4
Putative ABC transporter periplasmic binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yliB PE=4 SV=1	0.001	-0.131	-0.106	-0.139	0.246	0.216	4
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ygiN PE=4 SV=1	0.0012	-0.196	-0.188	-0.171	0.066	0.124	4
DNA protection during starvation protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dps PE=3 SV=3	0.0017	-0.088	-0.153	-0.086	0.151	0.258	4
Protein GrpE OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=grpE PE=3 SV=1	0.0018	-0.104	-0.214	-0.175	-0.018	0.097	4
Putative reductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ytfG PE=4 SV=1	0.0022	-0.154	-0.180	-0.192	0.042	0.019	4
Ribokinase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rbsK PE=3 SV=1	0.0029	-0.157	-0.134	-0.192	0.057	0.061	4
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yjeJ PE=4 SV=1	0.003	-0.075	-0.117	-0.209	0.023	0.095	4
Prolipoprotein diacylglyceryl transferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=lgt PE=3 SV=1	0.0042	-0.343	-0.186	-0.182	0.283	-0.051	4
Peptidyl-prolyl cis-trans isomerase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ppiA PE=3 SV=1	0.006	0.009	-0.004	-0.284	0.180	0.253	4
Putative periplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM1123 PE=4 SV=1	0.007	-0.097	-0.174	-0.075	0.163	0.194	4
Periplasmic trehalase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=treA PE=3 SV=1	0.012	-0.202	-0.239	-0.171	0.087	0.119	4
Adenylosuccinate synthetase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=purA PE=3 SV=2	0.014	-0.117	-0.125	-0.172	0.022	0.106	4
Mannose-specific PTS system protein IID OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=manZ PE=4 SV=1	0.015	-0.124	-0.127	-0.053	0.247	0.125	4

Malate dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=mdh PE=3 SV=2	0.017	-0.115	-0.122	-0.165	0.037	0.113	4
Glucose-1-phosphatase/inositol phosphatase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=agp PE=4 SV=1	0.017	-0.193	-0.222	-0.160	0.102	0.154	4
Polyamine aminopropyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=speE PE=3 SV=1	0.018	-0.128	-0.163	-0.156	-0.025	0.077	4
Flagellar basal-body rod protein FlgG OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=flgG PE=1 SV=1	0.019	-0.086	-0.148	-0.150	0.127	0.206	4
3-deoxy-D-manno-octulosonate 8-phosphate phosphatase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yrbI PE=4 SV=1	0.021	-0.157	-0.108	-0.073	0.059	0.216	4
Putative hemolysin-like protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ytfL PE=4 SV=1	0.022	-0.297	-0.037	-0.013	0.365	0.020	4
UPF0125 protein YfjF OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yfjF PE=3 SV=1	0.023	-0.177	-0.198	-0.049	0.015	0.060	4
Formyltetrahydrofolate deformylase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=purU PE=3 SV=1	0.025	-0.258	-0.153	-0.171	0.100	0.018	4
Acridine efflux pump OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=acrA PE=3 SV=1	0.028	-0.117	-0.146	-0.048	0.117	0.127	4
p-type ATPase family OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=zntA PE=3 SV=1	0.033	-0.169	-0.113	-0.087	0.072	0.054	4
dCTP deaminase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=dcd PE=3 SV=1	0.033	-0.362	-0.302	-0.357	0.131	0.179	4
NADH pyrophosphatase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=nudC PE=3 SV=1	0.035	-0.164	-0.107	-0.054	0.097	0.061	4
Putative enzyme with a TIM-barrel fold OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yggS PE=3 SV=1	0.041	-0.219	-0.122	-0.123	0.065	-0.032	4
Cyclic pyranopterin monophosphate synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=moaC PE=3 SV=3	0.042	-0.199	-0.189	-0.151	0.139	0.222	4
Putative gluconeogenesis factor OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ybhK PE=3 SV=1	0.045	-0.241	-0.149	-0.103	0.284	0.182	4
Small heat shock protein IbpA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ibpA PE=3 SV=1	0.05	-0.076	-0.099	-0.128	0.043	0.057	4

LPS-assembly protein LptD OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=imp PE=3 SV=1	< 0.0001	-0.193	-0.206	-0.167	0.224	0.259	5
Acyl-CoA thioesterase II OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tesB PE=4 SV=1	< 0.0001	-0.170	-0.213	-0.170	0.234	0.308	5
Flagellin OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fliC PE=1 SV=4	< 0.0001	-0.100	-0.099	-0.186	0.331	0.416	5
Protein ApaG OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=apaG PE=3 SV=2	< 0.0001	-0.197	-0.182	-0.176	0.194	0.280	5
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yiiM PE=4 SV=1	< 0.0001	-0.126	-0.133	-0.133	0.231	0.290	5
Flagellar L-ring protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=flgH PE=3 SV=1	< 0.0001	-0.242	-0.224	-0.246	0.413	0.425	5
Molybdenum cofactor biosynthesis protein B OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=moaB PE=3 SV=1	< 0.0001	-0.115	-0.141	-0.199	0.242	0.352	5
Flagellar basal body protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=flgF PE=3 SV=1	0.00016	-0.048	-0.194	-0.170	0.239	0.318	5
Putative transport protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yehZ PE=4 SV=1	0.00017	-0.118	-0.171	-0.088	0.223	0.307	5
Putative sulfur reduction protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ychN PE=4 SV=1	0.00083	-0.275	-0.215	-0.163	0.347	0.446	5
3-methyl-2-oxobutanoate hydroxymethyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=panB PE=3 SV=1	0.0014	-0.100	-0.169	-0.229	0.258	0.321	5
Regulator of ribonuclease activity A OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rraA PE=3 SV=1	0.012	-0.105	-0.159	-0.130	0.220	0.328	5
Putative aldo/keto reductase family OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM1676 PE=4 SV=1	0.047	-0.200	-0.112	-0.049	0.228	0.310	5
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ycgM PE=4 SV=1	0.043	0.122	0.218	-0.041	1.584	0.746	6
DNA-binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=stpA PE=3 SV=1	< 0.0001	-0.036	-0.002	-0.027	-0.804	-0.729	7
Tryptophan--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=trpS PE=3 SV=1	< 0.0001	-0.014	0.033	-0.035	-0.599	-0.582	7
30S ribosomal protein S12 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpsL PE=3 SV=2	< 0.0001	-0.057	-0.060	-0.078	-0.662	-0.704	7

Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ybgS PE=4 SV=1	0.013	0.167	0.218	0.045	-1.328	-0.947	7
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yceD PE=4 SV=1	0.015	0.098	0.041	0.023	-0.615	-0.641	7
Ribosome modulation factor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rmf PE=2 SV=1	0.016	-0.171	-0.102	0.077	-1.113	-1.160	7
50S ribosomal protein L31 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpmE PE=3 SV=1	0.041	-0.055	0.002	0.059	-0.869	-0.733	7
50S ribosomal protein L34 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpmH PE=3 SV=1	0.042	0.373	0.553	0.525	-1.134	-1.123	8
Glycine--tRNA ligase beta subunit OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=glyS PE=3 SV=3	< 0.0001	0.000	0.003	-0.045	-0.391	-0.427	9
Mannitol-1-phosphate 5-dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=mtlD PE=3 SV=1	< 0.0001	-0.143	-0.084	-0.069	-0.431	-0.420	9
Ribosome-binding ATPase YchF OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ychF PE=3 SV=1	< 0.0001	-0.069	-0.029	-0.070	-0.352	-0.381	9
Translation initiation factor IF-2 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=infB PE=3 SV=1	< 0.0001	0.037	-0.057	-0.007	-0.338	-0.435	9
Putative ABC transporter ATP-binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yjjK PE=4 SV=1	< 0.0001	-0.064	-0.038	-0.116	-0.433	-0.474	9
Sugar phosphatase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yidA PE=4 SV=1	< 0.0001	-0.008	-0.056	-0.004	-0.409	-0.385	9
Chaperone protein DnaJ OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dnaJ PE=3 SV=2	< 0.0001	-0.087	-0.045	-0.093	-0.399	-0.403	9
Succinate--CoA ligase [ADP-forming] subunit alpha OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sucD PE=3 SV=1	< 0.0001	-0.097	-0.045	-0.070	-0.390	-0.361	9
Asparagine synthetase B OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=asnB PE=4 SV=1	0.002	-0.117	-0.034	-0.120	-0.336	-0.418	9
Carbamoyl-phosphate synthase large chain OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=carB PE=3 SV=4	< 0.0001	0.034	0.032	0.042	-0.225	-0.290	10
Aldehyde-alcohol dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=adhE PE=3 SV=1	< 0.0001	0.051	0.043	0.027	-0.222	-0.300	10
GDP/GTP pyrophosphokinase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=relA PE=3 SV=1	< 0.0001	0.063	0.091	0.083	-0.229	-0.284	10

Aconitate hydratase B OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=acnB PE=3 SV=1	< 0.0001	0.087	0.052	0.060	-0.259	-0.306	10
Putative RNase R OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yhgF PE=4 SV=1	< 0.0001	0.002	0.043	-0.032	-0.188	-0.271	10
Alanine--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=alaS PE=3 SV=1	< 0.0001	0.036	0.036	0.017	-0.213	-0.229	10
Aspartate ammonia-lyase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aspA PE=3 SV=1	< 0.0001	0.034	0.003	0.057	-0.214	-0.285	10
Secreted effector protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sipA PE=4 SV=1	< 0.0001	0.090	0.061	-0.030	-0.135	-0.273	10
CDP-6-deoxy-delta-3,4-glucoseen reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rfbI PE=4 SV=1	< 0.0001	-0.018	-0.020	-0.048	-0.201	-0.241	10
Putative type II restriction enzyme, methylase subunit OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM4495 PE=4 SV=1	0.00011	0.000	0.003	0.021	-0.191	-0.314	10
Putative SAM-dependent methyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_1982 PE=4 SV=1	0.00011	-0.033	-0.045	0.041	-0.239	-0.243	10
Ribosomal RNA small subunit methyltransferase H OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rsmH PE=3 SV=1	0.00029	-0.010	-0.088	0.075	-0.173	-0.165	10
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ybeL PE=4 SV=1	0.00031	0.063	0.070	0.107	-0.273	-0.192	10
DNA methylase M OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hsdM PE=4 SV=1	0.00043	-0.037	0.010	-0.033	-0.238	-0.283	10
Threonine--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=thrS PE=3 SV=1	0.00048	0.011	-0.083	0.031	-0.216	-0.197	10
Putative serine protein kinase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yeaG PE=4 SV=1	0.00068	0.010	0.058	0.083	-0.220	-0.239	10
Fructose-1,6-bisphosphatase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glpX PE=3 SV=1	0.0017	0.023	-0.026	0.005	-0.232	-0.125	10
Putative rhodanese-related sulfurtransferases OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yibN PE=4 SV=1	0.0018	0.035	0.052	0.145	-0.241	-0.210	10
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=metE PE=3 SV=1	0.0024	-0.009	0.007	-0.059	-0.170	-0.266	10

Peptide chain release factor 2 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=prfB PE=3 SV=1	0.0039	0.073	-0.108	0.036	-0.206	-0.203	10
Autoinducer 2 import ATP-binding protein LsrA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=lsrA PE=1 SV=1	0.013	-0.011	0.017	-0.100	-0.196	-0.311	10
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yeaO PE=4 SV=1	0.013	-0.071	-0.047	0.028	-0.287	-0.148	10
ATP-dependent protease ATPase subunit HslU OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=hslU PE=3 SV=2	0.014	-0.003	-0.058	-0.004	-0.235	-0.185	10
DNA helicase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM2767 PE=3 SV=1	0.015	-0.071	0.057	-0.052	-0.218	-0.388	10
UTP--glucose-1-phosphate uridylyltransferase subunit GalF OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=galF PE=4 SV=1	0.032	-0.023	-0.101	0.024	-0.190	-0.137	10
3-phosphoshikimate 1-carboxyvinyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=aroA PE=3 SV=2	0.034	-0.067	-0.012	-0.132	-0.117	-0.232	10
DNA helicase II OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=uvrD PE=3 SV=2	0.035	0.005	-0.060	0.064	-0.141	-0.240	10
Phosphoglucosamine mutase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glmM PE=3 SV=1	0.036	-0.003	-0.053	0.032	-0.163	-0.112	10
50S ribosomal protein L2 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rplB PE=3 SV=1	< 0.0001	0.116	0.023	0.086	-0.467	-0.516	11
Negative modulator of initiation of replication OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=seqA PE=3 SV=1	< 0.0001	0.062	0.005	0.047	-0.447	-0.376	11
30S ribosomal protein S3 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsC PE=3 SV=1	0.0018	0.118	0.062	0.065	-0.351	-0.395	11
ATP-dependent RNA helicase RhlB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rhlB PE=3 SV=2	0.0044	0.239	-0.019	0.162	-0.347	-0.287	11
Lipopolysaccharide core heptose(I) kinase RfaP OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rfaP PE=3 SV=2	0.006	0.213	0.147	0.229	-0.210	-0.367	11
50S ribosomal protein L25 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplY PE=3 SV=1	0.031	0.145	0.089	0.184	-0.497	-0.369	11
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 /	0.00017	0.925	0.876	0.754	0.378	0.336	12

SGSC1412 / ATCC 700720) GN=STM4492 PE=4 SV=1							
Aconitate hydratase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=acnA PE=3 SV=1	< 0.0001	0.880	0.784	0.865	0.667	0.670	13
Ketodeoxygluconokinase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=kdgK PE=4 SV=1	0.00043	0.991	0.854	0.966	0.602	0.712	13
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM1624 PE=4 SV=1	0.024	0.908	0.867	0.850	0.768	0.869	13
Outer membrane protein A OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ompA PE=1 SV=1	< 0.0001	1.417	1.333	1.365	1.621	1.744	
Outer membrane protein C OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ompC PE=3 SV=1	< 0.0001	1.363	1.408	1.437	2.337	2.455	
Fatty acid oxidation complex subunit alpha OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fadB PE=3 SV=1	< 0.0001	1.505	1.526	1.503	1.208	1.245	
Outer membrane protein receptor / transporter for ferrichrome, colicin M, and phages T1, T5, and phi80 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fhuA PE=3 SV=1	< 0.0001	0.480	0.534	0.509	1.015	1.049	
Outer membrane channel protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tolC PE=4 SV=1	< 0.0001	0.845	0.771	0.786	1.124	1.205	
Acetyl-coenzyme A synthetase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=acs PE=1 SV=1	< 0.0001	1.314	1.285	1.265	1.057	1.104	
Outer membrane porin protein OmpD OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ompD PE=1 SV=1	< 0.0001	1.488	1.556	1.445	2.045	2.133	
cAMP-regulatory protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=crp PE=4 SV=1	< 0.0001	-1.063	-0.987	-1.008	-0.767	-0.855	
Outer membrane protein X OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ompX PE=4 SV=1	< 0.0001	1.203	1.202	1.196	1.924	2.015	
Long-chain fatty acid outer membrane transporter OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fadL PE=4 SV=1	< 0.0001	0.828	0.835	0.737	1.339	1.394	
1,4-alpha-glucan branching enzyme GlgB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glgB PE=3 SV=1	< 0.0001	0.281	0.304	0.295	0.553	0.590	
4-aminobutyrate aminotransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=gabT PE=3 SV=1	< 0.0001	1.373	1.377	1.319	1.509	1.466	

Acyl-coenzyme A dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fadE PE=2 SV=1	< 0.0001	1.425	1.432	1.499	1.579	1.475	
Aldehyde dehydrogenase B OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aldB PE=3 SV=1	< 0.0001	1.436	1.307	1.266	1.234	1.241	
Transcription termination/antitermination protein NusA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=nusA PE=3 SV=1	< 0.0001	-0.689	-0.683	-0.702	-0.723	-0.725	
Isocitrate lyase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aceA PE=4 SV=1	< 0.0001	1.017	0.972	0.991	1.305	1.343	
3-ketoacyl-CoA thiolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fadA PE=3 SV=1	< 0.0001	1.064	1.083	1.096	1.076	1.074	
Vitamin B12 transporter BtuB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=btuB PE=3 SV=2	< 0.0001	0.494	0.451	0.496	1.017	1.065	
Malate synthase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aceB PE=3 SV=1	< 0.0001	1.437	1.311	1.474	1.232	1.249	
30S ribosomal protein S16 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsP PE=3 SV=1	< 0.0001	-1.755	-1.603	-1.593	-1.826	-1.882	
Gamma-aminobutyraldehyde dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ydcW PE=3 SV=1	< 0.0001	0.855	0.763	0.800	0.970	1.033	
Superoxide dismutase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sodA PE=3 SV=1	< 0.0001	1.167	1.090	1.067	1.253	1.310	
Succinate-semialdehyde dehydrogenase I OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=gabD PE=3 SV=1	< 0.0001	1.473	1.383	1.409	1.592	1.620	
DNA-binding protein HU-alpha OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=hupA PE=3 SV=1	< 0.0001	-1.375	-1.352	-1.400	-2.037	-2.055	
50S ribosomal protein L20 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplT PE=3 SV=2	< 0.0001	-1.004	-0.829	-0.967	-1.279	-1.334	
Putative periplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ydgA PE=4 SV=1	< 0.0001	-0.547	-0.508	-0.524	-0.459	-0.491	
3-oxoacyl-[acyl-carrier-protein] synthase I OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fbaB PE=4 SV=1	< 0.0001	0.204	0.217	0.234	0.562	0.657	
RNA chaperone ProQ OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=proQ PE=3 SV=1	< 0.0001	-0.312	-0.326	-0.246	-0.594	-0.561	

30S ribosomal protein S21 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsU PE=3 SV=1	< 0.0001	-0.893	-0.875	-0.837	-1.395	-1.357	
Protein TolB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tolB PE=3 SV=1	< 0.0001	0.021	-0.044	0.017	0.248	0.324	
Flagellar P-ring protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=flgI PE=3 SV=1	< 0.0001	0.679	0.516	0.608	0.999	1.024	
Periplasmic serine endoprotease OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=degS PE=4 SV=1	< 0.0001	0.282	0.313	0.444	0.688	0.872	
Fumarate hydratase class I, aerobic OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fumA PE=3 SV=3	< 0.0001	-0.116	-0.202	-0.080	-0.412	-0.403	
2-oxoglutarate dehydrogenase decarboxylase component OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=sucA PE=4 SV=1	< 0.0001	0.205	0.165	0.191	-0.061	-0.128	
Esterase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yjfP PE=4 SV=1	< 0.0001	0.443	0.434	0.427	0.610	0.679	
3-ketoacyl-CoA thiolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fadI PE=3 SV=1	< 0.0001	1.613	1.532	1.567	1.753	1.585	
Putative ABC superfamily transport protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yrbC PE=4 SV=1	< 0.0001	0.499	0.449	0.534	0.824	0.875	
Transketolase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tktA PE=3 SV=1	< 0.0001	0.205	0.196	0.201	0.484	0.539	
Cyclic di-GMP-binding protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=bcsB PE=3 SV=1	< 0.0001	0.128	0.110	0.084	0.551	0.545	
50S ribosomal protein L17 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplQ PE=3 SV=1	< 0.0001	-0.364	-0.379	-0.338	-0.601	-0.604	
SapA-like protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_0360 PE=4 SV=1	< 0.0001	0.801	0.700	0.732	1.327	1.342	
Cold shock-like protein CspC OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cspC PE=4 SV=1	< 0.0001	-0.566	-0.579	-0.575	-0.419	-0.329	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ydfZ PE=4 SV=1	< 0.0001	-2.584	-2.484	-2.434	-2.687	-2.648	
50S ribosomal protein L13 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplM PE=3 SV=1	< 0.0001	-0.296	-0.278	-0.359	-0.784	-0.882	
UPF0304 protein YfbU OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfbU PE=3 SV=1	< 0.0001	0.123	0.138	0.141	0.405	0.506	

D-alanyl-D-alanine carboxypeptidase fraction A OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=dacA PE=3 SV=1	< 0.0001	-0.506	-0.476	-0.473	-0.489	-0.464	
Scaffolding protein for murein-synthesizing holoenzyme OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=mipA PE=4 SV=1	< 0.0001	-0.048	0.062	-0.040	0.692	0.675	
CTP synthase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pyrG PE=3 SV=1	< 0.0001	0.176	0.131	0.173	-0.222	-0.172	
Putative outer membrane lipoprotein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_2352 PE=4 SV=1	< 0.0001	-0.004	0.011	-0.026	0.539	0.564	
Arginine transport system OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=artI PE=3 SV=1	< 0.0001	-0.884	-0.840	-0.839	-0.639	-0.556	
3-oxoacyl-[acyl-carrier-protein] reductase FabG OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fabG PE=1 SV=1	< 0.0001	-0.041	-0.044	-0.076	0.257	0.355	
Cell division protein FtsZ OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ftsZ PE=3 SV=1	< 0.0001	-0.513	-0.495	-0.480	-0.564	-0.632	
Uridine phosphorylase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=udp PE=1 SV=2	< 0.0001	-0.005	-0.094	-0.013	0.302	0.340	
UPF0234 protein YajQ OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yajQ PE=3 SV=2	< 0.0001	-0.236	-0.289	-0.180	-0.646	-0.647	
Glucose-6-phosphate 1-dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=zwf PE=3 SV=1	< 0.0001	0.049	0.028	0.026	0.279	0.340	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yccJ PE=4 SV=1	< 0.0001	-0.695	-0.587	-0.680	-0.528	-0.553	
50S ribosomal protein L16 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rplP PE=3 SV=1	< 0.0001	-0.598	-0.579	-0.551	-0.938	-0.916	
Polyribonucleotide nucleotidyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pnp PE=1 SV=1	< 0.0001	0.098	0.077	0.086	0.301	0.331	
Long-chain-fatty-acid--CoA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fadD PE=3 SV=1	< 0.0001	0.612	0.660	0.567	0.609	0.638	
Carbamoyl-phosphate synthase small chain OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=carA PE=3 SV=1	< 0.0001	-0.402	-0.357	-0.337	-0.537	-0.469	
Outer membrane lipoprotein SlyB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=slyB PE=1 SV=1	< 0.0001	0.428	0.393	0.476	0.854	0.940	
30S ribosomal subunit S22 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262)	< 0.0001	-1.019	-1.037	-0.966	-2.083	-2.247	

GN=rpsV PE=4 SV=1							
Aspartate-semialdehyde dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=asd PE=3 SV=1	< 0.0001	0.071	0.086	0.028	0.389	0.459	
Integration host factor subunit alpha OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ihfA PE=3 SV=1	< 0.0001	-0.394	-0.331	-0.352	-0.909	-0.866	
Transcriptional regulator HU subunit beta OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hupB PE=3 SV=1	< 0.0001	-1.412	-1.411	-1.381	-1.775	-1.772	
Periplasmic oligopeptide-binding protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=oppA PE=1 SV=2	< 0.0001	0.358	0.271	0.280	0.449	0.494	
30S ribosomal protein S7 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsG PE=3 SV=1	< 0.0001	-0.329	-0.373	-0.363	-0.901	-0.961	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_2186 PE=4 SV=1	< 0.0001	-0.966	-0.902	-0.910	-1.030	-1.017	
Leucine--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=leuS PE=3 SV=1	< 0.0001	0.153	0.120	0.151	-0.180	-0.182	
Transcriptional regulatory protein RcsB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rcsB PE=3 SV=1	< 0.0001	-0.736	-0.708	-0.656	-0.829	-0.941	
Pyruvate formate lyase I, induced anaerobically OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pflB PE=4 SV=1	< 0.0001	0.172	0.142	0.131	-0.107	-0.149	
Outer membrane protease E OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pgtE PE=3 SV=2	< 0.0001	0.020	0.035	0.011	0.418	0.604	
50S ribosomal protein L1 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplA PE=3 SV=1	< 0.0001	-0.511	-0.420	-0.484	-0.628	-0.695	
Pyruvate dehydrogenase E1 component OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aceE PE=4 SV=1	< 0.0001	0.187	0.169	0.176	-0.133	-0.135	
Transketolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tktB PE=3 SV=1	< 0.0001	0.292	0.241	0.240	0.410	0.480	
Serine hydroxymethyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=glyA PE=1 SV=1	< 0.0001	-0.316	-0.298	-0.324	-0.346	-0.365	
Lipoyl synthase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=lipA PE=3 SV=1	< 0.0001	-0.796	-0.657	-0.769	-0.726	-0.845	

Glucose-6-phosphate isomerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pgi PE=3 SV=1	< 0.0001	0.116	0.117	0.099	0.432	0.457	
50S ribosomal protein L15 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rplO PE=3 SV=1	< 0.0001	-0.207	-0.270	-0.205	-0.417	-0.446	
Probable transcriptional regulatory protein YebC OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yebC PE=3 SV=1	< 0.0001	-0.723	-0.707	-0.706	-0.833	-0.758	
DNA-directed RNA polymerase subunit omega OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpoZ PE=3 SV=1	< 0.0001	-0.495	-0.401	-0.337	-0.897	-0.793	
Chemotaxis protein CheW OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cheW PE=1 SV=1	< 0.0001	-0.232	-0.225	-0.282	-0.087	-0.081	
Phosphoethanolamine transferase CptA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cptA PE=1 SV=1	< 0.0001	-0.647	-0.612	-0.598	-0.594	-0.689	
Malic enzyme OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=maeB PE=4 SV=1	< 0.0001	0.249	0.165	0.206	0.399	0.493	
Outer membrane secretin OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=invG PE=3 SV=1	< 0.0001	0.580	0.584	0.460	0.764	0.726	
Proline--tRNA ligase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=proS PE=3 SV=1	< 0.0001	0.055	0.087	0.016	0.266	0.308	
Putative stress-induced protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yicC PE=4 SV=1	< 0.0001	0.078	0.096	0.036	0.263	0.332	
Lon protease OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=lon PE=2 SV=1	< 0.0001	-0.091	-0.131	-0.100	-0.263	-0.313	
Phospholipase A1 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pldA PE=3 SV=1	< 0.0001	0.562	0.497	0.543	1.010	1.105	
Murein lipoprotein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=lpp PE=4 SV=1	< 0.0001	0.658	0.487	0.629	0.868	0.957	
Quinolinate phosphoribosyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=nadC PE=3 SV=1	< 0.0001	-1.166	-0.790	-1.110	-0.670	-0.706	
Oxidoreductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yghA PE=4 SV=1	< 0.0001	0.277	0.205	0.276	0.345	0.416	
N-methyl-L-tryptophan oxidase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=solA PE=3 SV=1	< 0.0001	0.020	-0.008	0.028	0.216	0.267	
Carbonic anhydrase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yadF PE=1 SV=1	< 0.0001	-0.022	0.010	-0.013	0.239	0.337	

RNA-binding protein YhbY OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yhbY PE=4 SV=1	< 0.0001	-0.138	-0.237	-0.147	-0.382	-0.397	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_2943 PE=4 SV=1	< 0.0001	0.283	0.213	0.337	0.644	0.808	
DNA-directed RNA polymerase subunit beta' OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpoC PE=1 SV=1	< 0.0001	0.208	0.203	0.210	0.134	0.107	
Exonuclease III OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=xthA PE=4 SV=1	< 0.0001	-0.198	-0.177	-0.190	-0.342	-0.311	
Putative dimethyl sulphoxide reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_1808 PE=4 SV=1	< 0.0001	-0.436	-0.426	-0.444	-0.707	-0.655	
Proline aminopeptidase P II OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pepP PE=4 SV=1	< 0.0001	0.402	0.359	0.432	0.320	0.322	
Putative outer membrane lipoprotein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yiaD PE=3 SV=1	< 0.0001	0.338	0.319	0.349	0.698	0.746	
Bacterioferritin OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=bfr PE=3 SV=1	< 0.0001	0.304	0.214	0.225	0.453	0.545	
50S ribosomal protein L5 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rplE PE=3 SV=1	< 0.0001	-0.335	-0.356	-0.375	-0.615	-0.628	
Glucans biosynthesis protein D OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=mdoD PE=3 SV=1	< 0.0001	0.327	0.344	0.211	0.589	0.589	
Purine nucleoside phosphorylase DeoD-type OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=deoD PE=3 SV=1	< 0.0001	0.062	0.034	0.028	0.319	0.430	
Succinate--CoA ligase [ADP-forming] subunit beta OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sucC PE=3 SV=1	< 0.0001	0.186	0.186	0.171	-0.134	-0.096	
30S ribosomal protein S19 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpsS PE=3 SV=2	< 0.0001	-0.374	-0.336	-0.323	-0.890	-0.856	
Methyl accepting chemotaxis protein II OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cheM PE=4 SV=1	< 0.0001	-0.221	-0.235	-0.118	0.035	-0.040	
Non-specific acid phosphatase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=phoN PE=3 SV=1	< 0.0001	-0.769	-0.729	-0.673	-0.711	-0.617	
Anti sigma E (Sigma 24) factor, negative regulator OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rseB PE=4 SV=1	< 0.0001	0.069	0.075	0.201	0.496	0.509	

Putative stress-response protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yjbJ PE=3 SV=1	< 0.0001	-0.266	-0.297	-0.309	-1.145	-1.132	
Phosphoglucomutase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pgm PE=1 SV=1	< 0.0001	-0.102	-0.081	-0.066	-0.323	-0.353	
PTS system glucose-specific EIICB component OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ptsG PE=1 SV=2	< 0.0001	-0.562	-0.472	-0.599	-0.450	-0.677	
Gifsy-1 prophage protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM1012 PE=4 SV=1	< 0.0001	-0.316	-0.370	-0.280	-0.627	-0.597	
Ribonuclease E OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rne PE=3 SV=1	< 0.0001	0.260	0.204	0.274	0.098	0.058	
Catabolic arginine decarboxylase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=adi PE=4 SV=1	< 0.0001	0.177	0.162	0.126	0.361	0.390	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yciE PE=4 SV=1	< 0.0001	0.815	0.673	0.678	0.767	0.839	
Putative periplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yhcB PE=4 SV=1	< 0.0001	0.203	0.150	0.226	0.363	0.400	
Lipid A palmitoyltransferase PagP OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pagP PE=3 SV=1	< 0.0001	-0.071	0.006	-0.087	0.552	0.642	
Nucleoid-associated protein YbaB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ybaB PE=3 SV=1	< 0.0001	-0.190	-0.277	-0.221	-0.617	-0.486	
Protein CsiD OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=csiD PE=3 SV=1	< 0.0001	1.202	1.302	1.401	1.601	1.427	
Glycine dehydrogenase (decarboxylating) OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=gcvP PE=3 SV=1	< 0.0001	-0.112	-0.063	-0.072	0.195	0.155	
Glucose-1-phosphate adenylyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glgC PE=3 SV=1	< 0.0001	0.440	0.391	0.460	0.650	0.599	
Oligoribonuclease OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=orn PE=3 SV=2	< 0.0001	-0.880	-0.867	-0.925	-0.349	-0.370	
50S ribosomal protein L22 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rplV PE=3 SV=1	< 0.0001	-0.280	-0.296	-0.270	-0.669	-0.606	
30S ribosomal protein S20 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpsT PE=3 SV=2	< 0.0001	-0.855	-0.806	-0.746	-2.246	-2.217	
Serine acetyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cysE PE=1 SV=1	< 0.0001	0.251	0.216	0.210	0.585	0.605	

Fumarate hydratase class I OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fumB PE=3 SV=1	< 0.0001	-0.084	-0.058	-0.095	-0.300	-0.350	
30S ribosomal protein S4 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpsD PE=1 SV=3	< 0.0001	-0.347	-0.367	-0.331	-0.692	-0.780	
Ribonuclease R OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=vacB PE=3 SV=1	< 0.0001	0.242	0.278	0.241	0.369	0.354	
Glyoxalase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yhbL PE=3 SV=1	< 0.0001	-0.565	-0.587	-0.645	-0.325	-0.386	
Putative outer membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ygiW PE=4 SV=1	< 0.0001	-0.717	-0.701	-0.716	-0.450	-0.468	
30S ribosomal protein S18 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsR PE=3 SV=1	< 0.0001	-1.423	-1.355	-1.392	-1.536	-1.656	
D-ribose transporter subunit RbsB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rbsB PE=4 SV=1	< 0.0001	0.012	-0.007	0.012	0.219	0.270	
Pyrimidine-specific ribonucleoside hydrolase RihA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rihA PE=3 SV=1	< 0.0001	0.006	-0.027	0.059	0.417	0.657	
ATP-dependent Clp protease ATP-binding subunit ClpX OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=clpX PE=3 SV=1	< 0.0001	-0.216	-0.222	-0.237	-0.384	-0.389	
Molybdate transporter periplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=modA PE=4 SV=1	< 0.0001	-0.022	0.003	-0.003	0.305	0.372	
Outer membrane protein assembly factor BamC OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nlpB PE=3 SV=1	< 0.0001	-0.191	-0.161	-0.216	-0.076	-0.030	
Dipeptidyl carboxypeptidase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dcp PE=1 SV=1	< 0.0001	0.285	0.333	0.206	0.657	0.717	
Aspartate carbamoyltransferase regulatory chain OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pyrI PE=3 SV=1	< 0.0001	-0.445	-0.470	-0.474	-0.287	-0.237	
Adenylate kinase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=adk PE=3 SV=1	< 0.0001	-0.485	-0.430	-0.459	-0.252	-0.262	
Lipoprotein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=metQ PE=3 SV=1	< 0.0001	0.070	-0.003	0.044	0.363	0.313	
NADP-specific glutamate dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=gdhA PE=3 SV=2	< 0.0001	0.008	0.103	-0.086	0.429	0.511	
Translation initiation factor IF-1 OS=*Salmonella* Typhimurium (strain LT2 /	< 0.0001	-0.959	-0.948	-0.925	-1.644	-1.699	

SGSC1412 / ATCC 700720) GN=infA PE=3 SV=2							
DNA gyrase subunit B OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=gyrB PE=1 SV=2	< 0.0001	-0.071	-0.068	-0.031	-0.263	-0.310	
Autonomous glycyl radical cofactor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfiD PE=3 SV=1	< 0.0001	-0.732	-0.736	-0.764	-0.856	-0.879	
Mannose-6-phosphate isomerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=manA PE=1 SV=2	< 0.0001	-0.197	-0.153	-0.146	-0.469	-0.459	
Glycogen synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=glgA PE=3 SV=2	< 0.0001	0.421	0.341	0.383	0.354	0.351	
Glycerol-3-phosphate dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glpD PE=3 SV=1	< 0.0001	0.352	0.343	0.370	0.244	0.360	
Cold shock protein CspA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cspA PE=3 SV=2	< 0.0001	-0.677	-0.584	-0.604	-0.414	-0.342	
Aspartate carbamoyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pyrB PE=3 SV=1	< 0.0001	0.103	0.021	0.044	0.356	0.443	
ClpXP protease specificity-enhancing factor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sspB PE=4 SV=1	< 0.0001	-0.193	-0.210	-0.160	-0.037	0.028	
GTP-binding elongation factor family protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=typA PE=4 SV=1	< 0.0001	0.107	0.065	0.104	-0.136	-0.170	
Trigger factor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tig PE=3 SV=1	< 0.0001	-0.207	-0.215	-0.180	-0.438	-0.448	
Phosphate acetyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pta PE=1 SV=1	< 0.0001	0.209	0.167	0.179	0.035	0.027	
Glucans biosynthesis protein G OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=mdoG PE=3 SV=1	< 0.0001	-0.048	-0.072	-0.044	0.224	0.288	
Pyruvate dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=poxB PE=3 SV=1	< 0.0001	0.312	0.306	0.309	0.071	0.104	
Peptidylprolyl isomerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cypD PE=4 SV=1	< 0.0001	-0.051	-0.058	-0.062	0.093	0.075	
Outer membrane lipoprotein RcsF OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rcsF PE=3 SV=1	< 0.0001	-0.329	-0.376	-0.445	-0.574	-0.545	
4-hydroxy-tetrahydrodipicolinate reductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dapB	< 0.0001	0.004	-0.063	0.093	0.373	0.451	

PE=3 SV=1							
Multifunctional acyl-CoA thioesterase I and protease I and lysophospholipase L1 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tesA PE=4 SV=1	< 0.0001	-0.813	-0.740	-0.750	-0.528	-0.415	
Pyridoxine/pyridoxamine 5'-phosphate oxidase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pdxH PE=3 SV=1	< 0.0001	-0.052	-0.056	0.005	0.210	0.271	
Phosphoribosylaminoimidazole-succinocarboxamide synthase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=purC PE=3 SV=1	< 0.0001	-0.431	-0.325	-0.315	-0.691	-0.617	
tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=mnmG PE=3 SV=1	< 0.0001	0.239	0.268	0.206	0.061	0.105	
NAD(P)H dehydrogenase (quinone) OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=wraB PE=3 SV=1	< 0.0001	-0.086	-0.079	-0.088	0.248	0.354	
50S ribosomal protein L24 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rplX PE=3 SV=1	< 0.0001	-0.586	-0.598	-0.582	-1.303	-1.280	
Cysteine--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cysS PE=3 SV=1	< 0.0001	0.246	0.225	0.264	0.130	0.150	
Putative nucleotide binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ygdH PE=4 SV=1	< 0.0001	0.184	0.210	0.161	0.306	0.269	
RNA-binding protein Hfq OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=hfq PE=1 SV=2	< 0.0001	-0.220	-0.244	-0.243	-0.523	-0.546	
5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=mtnN PE=3 SV=1	< 0.0001	-0.466	-0.447	-0.427	-0.284	-0.224	
Acyl carrier protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=acpP PE=3 SV=1	< 0.0001	-1.798	-1.721	-1.771	-1.293	-1.372	
Transcription elongation factor GreA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=greA PE=3 SV=1	< 0.0001	-0.387	-0.418	-0.435	-0.307	-0.237	
Glycine betaine/proline betaine transport system ATP-binding protein ProV OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=proV PE=2 SV=2	< 0.0001	-0.228	-0.238	-0.233	-0.355	-0.362	
30S ribosomal protein S13 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpsM PE=3 SV=3	< 0.0001	-0.133	-0.202	-0.093	-0.484	-0.470	

Enolase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=eno PE=3 SV=2	< 0.0001	-0.228	-0.239	-0.246	-0.483	-0.425	
Phosphopentomutase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=deoB PE=3 SV=1	< 0.0001	-0.134	-0.196	-0.101	-0.501	-0.500	
Outer membrane protein F OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ompF PE=1 SV=2	< 0.0001	1.164	1.085	1.068	1.772	1.945	
GTP cyclohydrolase-2 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ribA PE=3 SV=1	< 0.0001	-0.441	-0.413	-0.356	-0.277	-0.319	
Putative lipoprotein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ybjP PE=4 SV=1	< 0.0001	0.202	0.106	0.043	0.470	0.508	
DNA-binding protein H-NS OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=hns PE=1 SV=2	< 0.0001	-1.111	-1.022	-1.028	-1.149	-1.058	
Signal recognition particle protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ffh PE=3 SV=1	< 0.0001	-0.313	-0.279	-0.287	-0.613	-0.756	
Cell division protein ZapB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=zapB PE=3 SV=1	0.0001	-1.338	-1.136	-1.256	-0.732	-0.530	
Chemotaxis regulatory protein CheY OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cheY PE=4 SV=1	0.0001	-0.649	-0.581	-0.585	-0.869	-0.883	
Ribonuclease 3 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rnc PE=3 SV=1	0.00011	-0.340	-0.390	-0.235	-0.304	-0.200	
50S ribosomal protein L32 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpmF PE=3 SV=1	0.00011	-0.652	-0.663	-0.600	-1.770	-1.803	
30S ribosomal protein S1 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpsA PE=3 SV=1	0.00011	-0.318	-0.337	-0.324	-0.501	-0.477	
Putative ferripyochelin-binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yrdA PE=4 SV=1	0.00011	0.039	0.138	0.055	0.439	0.434	
Uronate isomerase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=uxaC PE=3 SV=1	0.00011	0.133	0.037	0.040	0.244	0.141	
Putative uroporphyrinogen III C-methyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hemX PE=4 SV=1	0.00011	-0.225	-0.252	-0.195	-0.108	-0.132	
Putative D-mannonate oxidoreductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_3796 PE=3 SV=1	0.00011	-0.279	-0.356	-0.304	-0.408	-0.506	
Translation inhibitor protein RaiA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfiA PE=4 SV=1	0.00011	-0.540	-0.481	-0.449	-1.093	-1.017	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yrbB PE=4 SV=1	0.00012	-0.195	-0.240	-0.151	-0.521	-0.388	

NADPH-dependent 7-cyano-7-deazaguanine reductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=queF PE=3 SV=1	0.00012	-0.194	-0.151	-0.151	0.002	-0.014	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_1428 PE=4 SV=1	0.00012	-0.127	-0.120	-0.122	-0.345	-0.445	
Translation initiation factor IF-3 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=infC PE=3 SV=1	0.00012	-0.266	-0.279	-0.189	-0.488	-0.553	
Ubiquinone/menaquinone biosynthesis C-methyltransferase UbiE OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ubiE PE=3 SV=1	0.00013	-0.128	-0.195	-0.154	-0.253	-0.234	
LexA repressor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=lexA PE=3 SV=1	0.00013	-0.403	-0.268	-0.344	-0.978	-0.871	
ATP synthase subunit alpha OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=atpA PE=3 SV=1	0.00013	0.211	0.173	0.176	0.224	0.267	
Virulence sensor histidine kinase PhoQ OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=phoQ PE=1 SV=1	0.00013	-1.166	-1.138	-1.074	-0.882	-1.148	
S-adenosylmethionine synthase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=metK PE=3 SV=1	0.00014	-0.682	-0.662	-0.675	-0.460	-0.416	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yciF PE=4 SV=1	0.00014	1.587	1.467	1.238	1.291	1.271	
Glutaredoxin 3 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=grxC PE=4 SV=1	0.00015	-0.463	-0.403	-0.367	-0.547	-0.476	
30S ribosomal protein S9 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsI PE=3 SV=1	0.00015	-0.448	-0.516	-0.464	-0.656	-0.599	
Catalase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=katE PE=3 SV=1	0.00016	0.244	0.210	0.238	0.489	0.503	
AMP nucleosidase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=amn PE=3 SV=1	0.00016	-0.054	-0.003	-0.032	0.396	0.453	
50S ribosomal protein L29 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpmC PE=3 SV=1	0.00016	-0.270	-0.357	-0.292	-1.737	-1.692	
NAD-dependent malic enzyme OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=maeA PE=3 SV=1	0.00017	0.161	0.140	0.106	0.028	-0.002	
Tricarboxylic transport OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_3361 PE=4 SV=1	0.00018	1.263	1.291	1.141	1.885	1.895	
tRNA 2-selenouridine synthase OS=*Salmonella* Typhimurium (strain LT2 /	0.00018	0.230	0.299	0.241	0.127	0.051	

SGSC1412 / ATCC 700720) GN=selU PE=1 SV=1							
Elongation factor Tu OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tuf_2 PE=3 SV=1	0.00018	-0.079	-0.149	-0.135	-0.381	-0.399	
Hydrogenase-3 accessory protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=hypB PE=4 SV=1	0.00018	-0.357	-0.358	-0.312	-0.401	-0.444	
Outer membrane protein W OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ompW PE=4 SV=1	0.00018	0.258	0.342	0.114	0.791	0.755	
Trypsin precursor cRAP	0.0002	0.156	0.270	0.200	0.466	0.456	
Probable Fe(2+)-trafficking protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yggX PE=1 SV=2	0.00022	-0.275	-0.277	-0.405	-0.410	-0.422	
Ribosomal protein S12 methylthiotransferase RimO OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yliG PE=3 SV=1	0.00022	-0.233	-0.199	-0.270	-0.529	-0.523	
Ribonucleoside-diphosphate reductase 1 subunit alpha OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nrdA PE=3 SV=1	0.00022	0.168	0.137	0.173	-0.078	-0.189	
NADH-quinone oxidoreductase subunit C/D OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=nuoC PE=3 SV=1	0.00023	0.187	0.205	0.212	0.209	0.161	
Transcription-repair-coupling factor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=mfd PE=3 SV=1	0.00023	0.098	0.195	0.141	0.037	-0.106	
GTP cyclohydrolase 1 type 2 homolog OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ybgI PE=3 SV=1	0.00024	-0.373	-0.282	-0.354	-0.251	-0.221	
Superoxide dismutase [Cu-Zn] 1 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=sodC1 PE=1 SV=1	0.00025	-0.517	-0.529	-0.515	-0.440	-0.406	
Protein CyaY OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cyaY PE=3 SV=1	0.00025	-1.051	-0.910	-1.173	-0.811	-0.818	
Cysteine desulfurase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sufS PE=3 SV=1	0.00025	0.384	0.445	0.383	0.410	0.660	
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=slsA PE=4 SV=1	0.00025	0.109	0.084	0.122	0.490	0.424	
Respiratory NADH dehydrogenase 2 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ndh PE=4 SV=1	0.00025	-0.217	-0.148	-0.238	-0.210	-0.304	
Short chain dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ucpA PE=4	0.00027	-0.159	-0.209	-0.161	-0.240	-0.186	

SV=1							
Curved DNA-binding protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cbpA PE=3 SV=1	0.00029	-0.194	-0.220	-0.185	0.003	0.028	
Magnesium-transporting ATPase, P-type 1 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=mgtB PE=1 SV=3	0.00029	-0.165	0.017	0.127	0.142	0.251	
Putative outer membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=slp PE=4 SV=1	0.00029	0.198	0.050	0.085	0.451	0.436	
Putative methyl-accepting chemotaxis protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM3216 PE=4 SV=1	0.00031	-0.096	-0.102	-0.081	0.123	-0.024	
Methionyl-tRNA formyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fmt PE=3 SV=3	0.00032	-0.145	-0.116	-0.075	-0.264	-0.259	
Putative DNA repair ATPase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_5393 PE=4 SV=1	0.00032	0.169	0.127	0.191	0.001	-0.035	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_4308 PE=4 SV=1	0.00033	-0.009	-0.073	0.026	0.278	0.277	
3-deoxy-manno-octulosonate cytidylyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=kdsB PE=3 SV=1	0.00033	0.079	0.067	0.037	0.187	0.291	
sn-glycerol-3-phosphate-binding periplasmic protein UgpB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ugpB PE=3 SV=1	0.00034	0.176	0.131	0.263	0.429	0.415	
Protein phosphatase CheZ OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cheZ PE=3 SV=1	0.00035	-0.367	-0.402	-0.366	-0.464	-0.383	
Putative oxidoreductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yhhX PE=4 SV=1	0.00036	-0.372	-0.378	-0.413	-0.557	-0.514	
ADP-dependent (S)-NAD(P)H-hydrate dehydratase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yjeF PE=3 SV=1	0.00036	-0.021	-0.006	0.022	0.359	0.417	
DNA helicase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=helD PE=3 SV=1	0.00036	0.637	0.531	0.598	0.446	0.322	
Oxygen-insensitive NADPH nitroreductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nfsA PE=3 SV=2	0.00036	-0.014	-0.026	0.001	0.281	0.343	
Pyruvate-flavodoxin oxidoreductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=nifJ PE=3 SV=1	0.00037	0.239	0.349	0.282	0.364	0.256	

Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_3005 PE=4 SV=1	0.00038	-0.737	-0.851	-0.702	-0.441	-0.372	
2-dehydro-3-deoxyphosphooctonate aldolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=kdsA PE=3 SV=1	0.00039	-0.121	-0.125	-0.156	-0.174	-0.114	
Phenylalanine--tRNA ligase alpha subunit OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pheS PE=3 SV=1	0.0004	0.145	0.097	0.170	0.181	0.231	
Protein deglycase YajL OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yajL PE=3 SV=2	0.00041	-0.366	-0.283	-0.384	-0.329	-0.487	
D-alanyl-D-alanine carboxypeptidase penicillin-binding protein 6a OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dacC PE=3 SV=1	0.00041	0.025	0.058	0.067	0.263	0.192	
Putative resistance protein MccF OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ycgQ PE=4 SV=1	0.00043	0.510	0.441	0.464	0.788	0.775	
Putative LysR family transcriptional regulator OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yeiE PE=4 SV=1	0.00045	0.165	0.204	-0.063	0.359	0.371	
DNA restriction enzyme OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=res PE=4 SV=1	0.00045	0.394	0.375	0.405	0.214	0.137	
Putative carbon starvation protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yjiY PE=4 SV=1	0.00045	-0.474	-0.397	-0.433	-0.297	-0.424	
Transcriptional regulator OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rob PE=4 SV=1	0.00047	-0.592	-0.524	-0.536	-0.438	-0.539	
Putative periplasmic binding transport protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fliY PE=3 SV=1	0.00047	-0.021	-0.063	-0.023	0.109	0.195	
UPF0325 protein YaeH OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yaeH PE=3 SV=1	0.00048	-0.396	-0.404	-0.393	-0.604	-0.568	
Putative aldo/keto reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ydhF PE=4 SV=1	0.00048	0.402	0.422	0.436	0.692	0.608	
Putative ABC transporter periplasmic binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yejA PE=4 SV=1	0.00049	0.565	0.487	0.475	0.786	0.789	
Soluble pyridine nucleotide transhydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=udhA PE=3 SV=1	0.00049	0.187	0.184	0.266	0.125	0.160	
ECA polysaccharide chain length modulation protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 /	0.00051	0.002	-0.037	0.043	0.430	0.207	

ATCC 700720) GN=wzzE PE=3 SV=2							
Polyphosphate kinase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ppk PE=3 SV=3	0.00052	0.329	0.240	0.281	0.151	0.126	
Cytoplasmic glycerophosphodiester phosphodiesterase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ugpQ PE=4 SV=1	0.00055	0.038	0.063	0.044	-0.178	-0.154	
Glutamate dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM1795 PE=3 SV=1	0.00056	0.101	0.106	0.141	0.257	0.308	
Putative hydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_1852 PE=4 SV=1	0.00056	-0.937	-0.783	-0.783	-0.360	-0.425	
Cysteine/glutathione ABC transporter membrane/ATP-binding component OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cydC PE=4 SV=1	0.00056	-0.034	0.064	0.028	0.579	0.529	
Putative hydrolase of the HAD superfamily OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ybiV(1) PE=4 SV=1	0.0006	-0.164	-0.162	-0.353	-0.347	-0.446	
Dihydrolipoyl dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=lpdA PE=4 SV=1	0.00061	-0.135	-0.164	-0.121	-0.216	-0.232	
Putative outer membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ytfM PE=4 SV=1	0.00062	0.004	0.005	-0.013	0.432	0.418	
Lysine-N-methylase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fliB PE=3 SV=1	0.00062	-0.066	-0.096	-0.149	-0.310	-0.359	
Ribosome maturation factor RimM OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rimM PE=3 SV=1	0.00064	-0.268	-0.313	-0.324	-0.546	-0.540	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=psiF PE=4 SV=1	0.00064	-0.549	-0.543	-0.527	-0.728	-0.683	
30S ribosomal protein S5 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsE PE=3 SV=1	0.00065	-0.139	-0.148	-0.144	-0.453	-0.437	
Glutamate/aspartate import solute-binding protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=gltI PE=3 SV=3	0.00066	0.131	0.127	0.129	0.440	0.614	
Glucose-1-phosphate thymidylyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rfbA PE=3 SV=1	0.00067	-0.340	-0.257	-0.360	-0.236	-0.305	
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ybbK PE=4 SV=1	0.00068	0.214	0.232	0.257	0.387	0.402	

Succinate dehydrogenase iron-sulfur subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sdhB PE=3 SV=1	0.00072	-0.458	-0.440	-0.406	-0.544	-0.516	
Glutathionine S-transferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=gst PE=4 SV=1	0.00073	0.525	0.436	0.480	0.193	0.242	
Isoleucine--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ileS PE=3 SV=1	0.00075	0.196	0.167	0.194	0.153	0.119	
Putative glycerol-3-phosphate regulon repressor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yihW PE=4 SV=1	0.00075	0.143	0.076	0.191	0.282	0.332	
tRNA modification GTPase MnmE OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=trmE PE=3 SV=1	0.00075	0.029	-0.026	0.062	0.147	0.187	
Acetyltransferase component of pyruvate dehydrogenase complex OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aceF PE=3 SV=1	0.00077	-0.237	-0.237	-0.230	-0.192	-0.151	
Putative excinuclease ATPase subunit OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM2746 PE=4 SV=1	0.00081	0.278	0.259	0.279	0.073	-0.048	
Ketol-acid reductoisomerase (NADP(+)) OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ilvC PE=3 SV=1	0.00081	-0.226	-0.244	-0.227	-0.339	-0.333	
Fumarate and nitrate reduction regulatory protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fnr PE=3 SV=1	0.00083	-0.755	-0.479	-0.660	0.008	-0.313	
Rare lipoprotein A OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rlpA PE=3 SV=1	0.00084	-0.033	0.014	-0.016	0.274	0.226	
NH(3)-dependent NAD(+) synthetase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nadE PE=1 SV=1	0.00085	-0.134	-0.179	-0.146	-0.265	-0.200	
Pectinesterase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ybhC PE=4 SV=1	0.00086	0.016	0.018	-0.047	0.339	0.441	
Ribosomal RNA large subunit methyltransferase I OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yccW PE=3 SV=1	0.00086	0.153	0.083	0.174	0.266	0.384	
Aminopeptidase N OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pepN PE=4 SV=1	0.00087	0.099	0.084	0.088	-0.035	-0.057	
Phenylalanine--tRNA ligase beta subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pheT PE=3 SV=1	0.00088	0.233	0.147	0.204	0.025	0.001	
UPF0047 protein YjbQ OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yjbQ PE=3 SV=1	0.00091	0.112	0.054	0.108	0.271	0.422	

N-succinylglutamate 5-semialdehyde dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=astD PE=3 SV=1	0.00096	1.131	1.068	1.050	1.006	0.993	
Putative periplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_1578 PE=4 SV=1	0.00097	-1.178	-1.071	-1.097	-0.597	-0.654	
Virulence membrane protein PAGC OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pagC PE=4 SV=1	0.00099	1.507	1.669	1.727	2.748	2.344	
Valine--tRNA ligase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=valS PE=3 SV=1	0.001	0.011	0.018	0.005	0.147	0.103	
50S ribosomal protein L10 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplJ PE=3 SV=2	0.001	-0.769	-0.738	-0.761	-0.702	-0.748	
Alcohol dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=adhP PE=4 SV=1	0.001	-0.194	-0.194	-0.242	-0.196	-0.170	
Site-determining protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=minD PE=3 SV=1	0.001	-0.075	-0.095	-0.060	-0.295	-0.298	
Serine endoprotease OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=degQ PE=4 SV=1	0.001	-0.276	-0.314	-0.247	-0.236	-0.109	
Pyridoxine 5'-phosphate synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pdxJ PE=3 SV=2	0.001	-0.010	-0.059	0.044	0.246	0.204	
Methyl-accepting chemotaxis citrate transducer OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tcp PE=3 SV=1	0.001	-0.194	-0.253	-0.268	0.011	-0.136	
Pseudouridine synthase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rluC PE=3 SV=1	0.001	-0.175	-0.204	-0.120	-0.366	-0.268	
Putative Zn-dependent peptidase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yhjJ PE=4 SV=1	0.001	-0.414	-0.449	-0.431	-0.301	-0.387	
Bifunctional protein FolD OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=folD PE=3 SV=1	0.001	-0.111	-0.160	-0.061	-0.363	-0.430	
Putative phosphatase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM3595 PE=4 SV=1	0.001	-1.737	-1.730	-1.308	-1.392	-1.534	
Putative aldo-keto reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tas PE=4 SV=1	0.001	0.084	0.115	0.025	0.401	0.299	
Flavin prenyltransferase UbiX OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ubiX PE=3 SV=1	0.001	0.077	0.190	0.088	0.514	0.512	
Non-specific ribonucleoside hydrolase RihC OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rihC PE=3 SV=1	0.001	1.135	1.136	1.160	1.417	1.484	

Acetolactate synthase isozyme 3 small subunit OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ilvH PE=3 SV=2	0.001	-0.894	-0.877	-0.914	-0.386	-0.427	
UDP-3-O-acyl-N-acetylglucosamine deacetylase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=lpxC PE=3 SV=1	0.001	0.216	0.225	0.174	-0.058	-0.034	
2,4-dieonyl-CoA reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fadH PE=4 SV=1	0.001	1.307	1.349	1.358	1.145	1.241	
Lipopolysaccharide assembly protein B OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yciM PE=3 SV=1	0.001	0.408	0.349	0.362	0.221	0.216	
Tol protein, membrane-spanning inner membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tolQ PE=3 SV=1	0.001	-0.032	-0.076	-0.086	0.297	0.236	
50S ribosomal protein L3 glutamine methyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfcB PE=3 SV=1	0.001	0.275	0.181	0.227	-0.116	-0.048	
Protease II OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ptrB PE=4 SV=1	0.001	0.702	0.682	0.727	0.868	0.959	
Glutaredoxin 1 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=grxA PE=3 SV=1	0.001	-0.840	-0.712	-0.756	-0.793	-0.835	
Lipoprotein NlpI OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=nlpI PE=4 SV=1	0.001	0.022	-0.058	0.035	0.457	0.557	
Phosphate import ATP-binding protein PstB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pstB PE=3 SV=1	0.001	-0.352	-0.323	-0.338	-0.356	-0.405	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ygaD PE=4 SV=1	0.001	0.286	0.287	0.471	1.059	1.086	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yecE PE=4 SV=1	0.0011	-0.509	-0.650	-0.538	-0.271	-0.504	
Pyrroline-5-carboxylate reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=proC PE=3 SV=1	0.0011	-0.253	-0.220	-0.253	-0.376	-0.348	
Ribonuclease I OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rna PE=3 SV=1	0.0012	-0.464	-0.448	-0.516	-0.396	-0.237	
Glycerol dehydrogenase, NAD OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=gldA PE=4 SV=1	0.0012	-0.341	-0.388	-0.239	-0.371	-0.356	
Plasmid partition protein B OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=parB PE=3 SV=1	0.0013	-0.347	-0.348	-0.334	-0.421	-0.336	

50S ribosomal protein L3 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rplC PE=3 SV=1	0.0013	0.233	0.101	0.222	-0.172	-0.140	
2-methylisocitrate lyase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=prpB PE=1 SV=3	0.0013	0.994	0.919	1.244	2.099	1.809	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM1672 PE=4 SV=1	0.0013	0.308	0.159	0.229	0.482	0.474	
Glutamine--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=glnS PE=3 SV=3	0.0013	0.102	0.089	0.091	-0.183	-0.155	
Porphobilinogen deaminase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hemC PE=3 SV=1	0.0013	-0.132	-0.214	-0.141	-0.027	-0.007	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=elaA PE=4 SV=1	0.0014	-0.658	-0.569	-0.655	-0.450	-0.558	
Acetyl-CoA carboxylase biotin carboxylase subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=accC PE=4 SV=1	0.0014	0.089	0.082	0.106	0.141	0.177	
Signal recognition particle receptor FtsY OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ftsY PE=3 SV=1	0.0015	-0.265	-0.205	-0.218	-0.160	-0.115	
Putative hydrolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ybeM PE=4 SV=1	0.0015	0.247	0.271	0.360	0.858	0.910	
Virulence transcriptional regulatory protein PhoP OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=phoP PE=1 SV=1	0.0016	-0.236	-0.223	-0.175	-0.149	-0.152	
Phosphoesterase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yfcE PE=3 SV=1	0.0016	0.061	-0.095	-0.106	0.317	0.172	
Ferritin OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ftn PE=3 SV=1	0.0016	0.064	-0.003	0.003	0.296	0.363	
Succinylornithine transaminase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=argD_1 PE=3 SV=1	0.0017	1.357	1.420	1.313	2.059	2.472	
Resistance to complement killing OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rcK PE=4 SV=1	0.0017	1.121	1.204	1.260	1.593	1.655	
Putative NADP-dependent oxidoreductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yncB PE=4 SV=1	0.0017	0.176	0.325	0.247	0.419	0.390	
Putative transcriptional regulator of two-component regulator protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yfhA PE=4 SV=1	0.0017	-0.149	-0.213	-0.217	-0.253	-0.274	
NADH-quinone oxidoreductase subunit I OS=*Salmonella* Typhimurium (strain LT2 /	0.0019	0.455	0.441	0.379	0.321	0.289	

SGSC1412 / ATCC 700720) GN=nuoI PE=3 SV=1							
Putative IclR family transcriptional repressor OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=kdgR PE=4 SV=1	0.002	0.180	0.170	0.138	0.317	0.248	
Putative ABC transporter ATPase component OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ybiT PE=4 SV=1	0.002	-0.183	-0.239	-0.163	-0.357	-0.483	
4-alpha-glucanotransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=malQ PE=3 SV=1	0.002	0.267	0.509	0.428	0.187	0.039	
Tellurite resistance protein TehB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=tehB PE=4 SV=1	0.002	-0.192	-0.235	-0.199	-0.460	-0.457	
2-methylcitrate synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=prpC PE=1 SV=2	0.002	1.118	1.050	1.235	1.280	1.304	
Riboflavin biosynthesis protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ribF PE=3 SV=1	0.002	-0.647	-0.609	-0.611	-0.394	-0.597	
Succinate dehydrogenase iron-sulfur subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=frdB PE=3 SV=1	0.0021	-0.387	-0.406	-0.401	-0.577	-0.621	
DNA-binding transcriptional repressor GlpR OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glpR PE=4 SV=1	0.0021	0.126	0.149	0.181	0.325	0.261	
ATP-binding subunit of serine protease OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=clpA PE=3 SV=1	0.0021	-0.091	-0.100	-0.066	-0.197	-0.211	
Glutamine ABC transporter periplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glnH PE=3 SV=1	0.0021	-0.390	-0.256	-0.304	-0.139	-0.056	
FtsH protease regulator HflK OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hflK PE=4 SV=1	0.0021	0.096	0.101	0.159	-0.045	-0.076	
Probable cytosol aminopeptidase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pepA PE=3 SV=1	0.0022	-0.162	-0.165	-0.147	-0.010	-0.031	
Isoaspartyl dipeptidase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=iadA PE=3 SV=1	0.0022	0.093	0.305	0.105	0.370	0.503	
Delta-aminolevulinic acid dehydratase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hemB PE=3 SV=1	0.0023	0.004	0.108	-0.039	0.622	0.531	
4-hydroxy-tetrahydrodipicolinate synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dapA PE=1 SV=1	0.0023	-0.305	-0.348	-0.308	-0.173	-0.101	

Pantothenate synthetase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=panC PE=1 SV=1	0.0024	-0.058	-0.046	-0.112	0.203	0.156	
Putative glutathionylspermidine synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ygiC PE=4 SV=1	0.0024	0.165	0.127	0.127	0.457	0.470	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ycfF PE=4 SV=1	0.0024	0.096	0.028	0.182	0.596	0.516	
Mannonate dehydratase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=uxuA PE=3 SV=1	0.0024	0.203	0.217	0.212	-0.032	-0.011	
SsrA-binding protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=smpB PE=3 SV=2	0.0025	-0.882	-0.861	-0.823	-0.763	-0.816	
Rhamnosyl transferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rfbN PE=4 SV=1	0.0025	0.547	0.446	0.422	0.187	0.096	
Adenosine deaminase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=add PE=3 SV=1	0.0026	-0.199	-0.234	-0.211	-0.167	-0.117	
Protoporphyrinogen oxidase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hemG PE=4 SV=1	0.0026	-0.374	-0.419	-0.316	-0.281	-0.384	
Gamma-glutamyl phosphate reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=proA PE=3 SV=1	0.0026	-0.218	-0.270	-0.151	-0.322	-0.384	
L-serine deaminase I/L-threonine deaminase I OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sdaA PE=4 SV=1	0.0027	-0.060	-0.035	-0.071	-0.267	-0.268	
30S ribosomal protein S8 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rpsH PE=3 SV=2	0.0029	-0.286	-0.413	-0.327	-0.408	-0.424	
4-hydroxythreonine-4-phosphate dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pdxA_1 PE=3 SV=1	0.0029	0.352	0.304	0.473	0.175	0.234	
Putative periplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ydgH PE=4 SV=1	0.003	-0.549	-0.546	-0.517	-0.784	-0.693	
Fatty acid oxidation complex subunit alpha OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=fadJ PE=3 SV=1	0.003	0.657	0.778	0.715	0.958	0.613	
Periplasmic chaperone OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hlpA PE=3 SV=1	0.003	-1.676	-1.474	-1.626	-1.455	-1.460	
Glutamate--cysteine ligase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=gshA PE=3 SV=1	0.003	0.303	0.225	0.274	0.435	0.528	
Putative sugar nucleotide epimerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yfcH	0.003	0.378	0.398	0.455	0.626	0.561	

PE=4 SV=1							
Dipeptide transport protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=dppA PE=4 SV=1	0.003	0.596	0.665	0.667	0.798	0.965	
Putative phosphatase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfbT PE=4 SV=1	0.003	-0.817	-0.652	-0.807	-0.598	-0.652	
PTS family sugar specific enzyme III for cellobiose, arbutin, and salicin OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=celC PE=4 SV=1	0.003	-0.187	-0.293	-0.166	-0.238	-0.017	
30S ribosomal protein S17 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsQ PE=3 SV=1	0.003	1.143	0.954	1.086	0.738	0.896	
RNA polymerase-binding transcription factor DksA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dksA PE=3 SV=1	0.0031	-0.326	-0.275	-0.328	-0.243	-0.185	
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=elaB PE=4 SV=1	0.0032	0.793	0.613	0.750	0.764	0.858	
Transcriptional regulator PhoB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=phoB PE=4 SV=1	0.0034	-0.506	-0.441	-0.409	-0.387	-0.436	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yciN PE=4 SV=1	0.0038	-0.259	-0.264	-0.215	-0.234	-0.185	
Putative phosphosugar isomerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM3601 PE=4 SV=1	0.004	-0.038	-0.153	-0.134	-0.320	-0.230	
Cobalt-precorrin-2 C(20)-methyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cbiL PE=1 SV=2	0.004	0.156	0.105	0.148	0.406	0.448	
Bifunctional glutathionylspermidine amidase/glutathionylspermidine synthetase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=gsp PE=4 SV=1	0.004	0.172	0.228	0.116	-0.041	0.001	
Galactokinase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=galK PE=3 SV=2	0.004	-0.213	-0.164	-0.224	-0.311	-0.314	
HTH-type transcriptional regulator CysB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cysB PE=1 SV=1	0.004	0.298	0.193	0.321	0.150	0.218	
Arginine transport system component OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=artJ PE=3 SV=1	0.004	0.286	0.375	0.440	0.710	0.688	
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yhjG PE=4 SV=1	0.0042	0.188	0.167	0.209	0.439	0.351	

NAD(P) transhydrogenase subunit alpha OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pntA PE=3 SV=1	0.0042	-0.238	-0.233	-0.194	-0.047	-0.171	
Putative nucleotide-binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yebR PE=4 SV=1	0.0042	-0.312	-0.208	-0.351	-0.232	-0.254	
Ribosomal protein L11 methyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=prmA PE=3 SV=1	0.0043	-0.522	-0.509	-0.510	-0.466	-0.431	
ADP-L-glycero-D-manno-heptose-6-epimerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=hldD PE=3 SV=1	0.0043	0.000	0.006	-0.091	0.133	0.246	
ATP-dependent RNA helicase DeaD OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=deaD PE=3 SV=1	0.0044	-0.135	-0.172	-0.118	-0.370	-0.428	
50S ribosomal protein L18 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplR PE=3 SV=1	0.0047	-0.417	-0.424	-0.382	-0.803	-0.761	
Hypoxanthine-guanine phosphoribosyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hpt PE=4 SV=1	0.0048	-0.564	-0.492	-0.567	-0.625	-0.754	
Putative glucose-6-phosphate 1-epimerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yeaD PE=1 SV=1	0.005	-0.298	-0.252	-0.262	-0.416	-0.367	
Transcriptional regulator sirC OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=sirC PE=4 SV=1	0.005	-1.228	-1.017	-1.164	-0.513	-0.743	
RNA polymerase sigma-54 factor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpoN PE=3 SV=1	0.005	0.076	0.103	0.153	-0.094	-0.078	
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yedE PE=4 SV=1	0.005	-1.634	-1.532	-1.627	-1.099	-1.375	
HTH-type transcriptional regulator CueR OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cueR PE=3 SV=1	0.005	-0.479	-0.467	-0.346	-0.706	-0.696	
Uncharacterized protein YciH OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yciH PE=3 SV=1	0.005	-0.487	-0.423	-0.439	-0.584	-0.689	
Beta-barrel assembly-enhancing protease OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=bepA PE=3 SV=1	0.0056	-0.351	-0.426	-0.167	-0.195	-0.120	
50S ribosomal protein L4 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplD PE=1 SV=1	0.0057	-0.305	-0.292	-0.310	-0.633	-0.683	

2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=dapD PE=3 SV=1	0.0059	-0.057	-0.077	-0.044	0.203	0.247	
Citrate synthase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=gltA PE=3 SV=1	0.006	0.270	0.250	0.289	0.276	0.278	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=phnA PE=4 SV=1	0.006	-0.794	-0.802	-0.776	-0.946	-0.878	
Peptide methionine sulfoxide reductase MsrB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=msrB PE=3 SV=1	0.006	-0.245	-0.339	-0.277	-0.327	-0.176	
Chemotaxis protein methyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cheR PE=4 SV=1	0.0068	-0.410	-0.443	-0.373	-0.268	-0.337	
UPF0246 protein YaaA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yaaA PE=3 SV=1	0.007	-0.109	-0.132	-0.112	-0.345	-0.305	
S-adenosylmethionine decarboxylase proenzyme OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=speD PE=3 SV=1	0.007	-0.433	-0.460	-0.475	-0.921	-0.939	
Putative alcohol dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yqhD PE=4 SV=1	0.0075	-0.045	-0.042	-0.012	-0.330	-0.276	
Anti-adapter protein IraP OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yaiB PE=3 SV=1	0.01	-0.405	-0.412	-0.431	-0.551	-0.520	
Periplasmic nitrate reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=napA PE=3 SV=1	0.01	-0.212	-0.198	-0.360	-0.290	-0.355	
Nucleoid-associated protein YejK OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yejK PE=3 SV=1	0.01	0.517	0.479	0.488	0.221	0.354	
Response regulator in two-component regulatory system with NarQ (Or NarX) OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=narP PE=4 SV=1	0.01	-0.707	-0.599	-0.662	-0.202	-0.300	
Bifunctional aspartate kinase II/homoserine dehydrogenase II OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=metL PE=4 SV=1	0.011	0.185	0.176	0.186	0.070	0.100	
Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=accA PE=3 SV=2	0.011	-0.180	-0.135	-0.237	-0.001	-0.028	
Type I restriction enzyme EcoKI subunit R OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hsdR PE=4 SV=1	0.011	0.242	0.257	0.317	0.188	0.000	

D-lactate dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=dld PE=4 SV=1	0.011	-0.051	-0.015	-0.079	0.236	0.257	
Formate dehydrogenase alpha subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fdoG PE=3 SV=1	0.011	0.379	0.413	0.418	0.173	0.222	
Aminotransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfdZ PE=4 SV=1	0.011	0.202	0.143	0.264	0.390	0.337	
Pyruvate formate-lyase-activating enzyme OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pflA PE=3 SV=1	0.011	-0.125	-0.137	-0.184	-0.505	-0.525	
Sensor protein QseC OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=qseC PE=3 SV=1	0.012	0.245	0.514	0.457	0.915	0.681	
Biopolymer transport protein ExbB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=exbB PE=3 SV=1	0.012	-0.280	-0.262	-0.163	-0.253	-0.227	
1,4-dihydroxy-2-naphthoyl-CoA hydrolase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=menI PE=3 SV=1	0.012	-0.083	-0.040	-0.071	0.188	0.225	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM2475 PE=4 SV=1	0.012	-0.480	-0.577	-0.409	-0.804	-0.836	
Nitrate reductase 1 alpha subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=narG PE=3 SV=1	0.013	0.109	0.089	0.179	0.094	0.157	
6,7-dimethyl-8-ribityllumazine synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ribH PE=1 SV=1	0.013	0.397	0.300	0.398	0.682	0.840	
Putative ABC transporter ATP-binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yheS PE=4 SV=1	0.013	0.159	0.247	0.119	-0.060	-0.183	
Putative gntR family regulatory protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM1541 PE=4 SV=1	0.013	-0.238	-0.087	-0.168	0.008	-0.136	
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yebE PE=4 SV=1	0.013	0.582	0.556	0.706	0.767	0.674	
30S ribosomal protein S10 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rpsJ PE=3 SV=1	0.014	0.251	0.207	0.242	-0.041	0.013	
NADH-quinone oxidoreductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=nuoG PE=3 SV=1	0.014	0.025	0.020	0.015	-0.107	-0.136	
Oligopeptidase A OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=prlC PE=3 SV=1	0.014	0.183	0.212	0.149	0.044	0.014	

Hydrogenase 2 large subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=hybC PE=3 SV=1	0.014	-0.394	-0.293	-0.298	-0.232	-0.435	
UPF0214 protein YfeW OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yfeW PE=3 SV=1	0.014	0.072	0.070	0.162	0.832	0.742	
Diaminopimelate decarboxylase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=lysA PE=3 SV=1	0.014	-0.068	0.042	0.046	0.519	0.253	
DNA-binding transcriptional regulator LysR OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=lysR PE=4 SV=1	0.014	0.008	0.104	0.058	0.381	0.394	
Pyruvate kinase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pykA PE=3 SV=1	0.015	-0.049	-0.039	-0.070	0.074	0.028	
DNA topoisomerase 4 subunit B OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=parE PE=1 SV=1	0.015	-0.378	-0.241	-0.343	-0.410	-0.535	
Ribosome-binding factor A OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rbfA PE=3 SV=1	0.015	-0.429	-0.331	-0.311	-0.514	-0.532	
UDP-N-acetylglucosamine 2-epimerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=wecB PE=3 SV=1	0.015	0.155	0.107	0.134	0.290	0.118	
UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfbE PE=3 SV=1	0.015	-0.112	-0.033	-0.012	0.089	0.155	
Putative cytoplasmic protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfcZ PE=4 SV=1	0.015	-0.681	-0.694	-0.557	-0.244	-0.247	
Chaperone protein ClpB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=clpB PE=3 SV=1	0.016	0.096	0.085	0.089	0.114	0.152	
Putative inner membrane lipoprotein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_3105 PE=4 SV=1	0.016	0.110	0.279	0.063	0.759	0.455	
Flagella synthesis protein FlgN OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=flgN PE=1 SV=1	0.016	-0.333	-0.377	-0.215	-0.548	-0.501	
Phosphoenolpyruvate-protein phosphotransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ptsI PE=1 SV=1	0.017	0.010	0.026	0.011	-0.108	-0.134	
50S ribosomal protein L21 OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rplU PE=3 SV=1	0.017	0.179	0.134	0.190	-0.154	-0.070	
Branched-chain-amino-acid aminotransferase OS=*Salmonella*	0.017	-0.187	-0.219	-0.183	-0.080	-0.013	

Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ilvE PE=1 SV=2							
3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fabZ PE=3 SV=1	0.017	-0.093	0.007	-0.006	0.307	0.453	
Protein FdhE OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fdhE PE=3 SV=1	0.017	-0.287	-0.320	-0.294	-0.321	-0.476	
UPF0115 protein YfcN OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfcN PE=3 SV=1	0.017	-0.426	-0.405	-0.512	-0.640	-0.568	
Erythronate-4-phosphate dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pdxB PE=3 SV=1	0.018	0.149	0.150	0.156	0.030	0.069	
CDP-diacylglycerol--serine O-phosphatidyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pssA PE=3 SV=1	0.018	0.604	0.461	0.665	0.242	0.260	
Gluconate operon transcriptional repressor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=gntR PE=4 SV=1	0.018	0.007	0.040	0.002	0.208	0.202	
Cell division protein FtsE OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ftsE PE=4 SV=1	0.018	0.158	0.291	0.100	0.425	0.437	
Spermidine/putrescine import ATP-binding protein PotA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=potA PE=3 SV=2	0.018	-0.499	-0.399	-0.416	-0.163	-0.250	
Elongation factor P-like protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yeiP PE=3 SV=1	0.018	-0.212	-0.231	-0.231	-0.463	-0.375	
3'(2'),5'-bisphosphate nucleotidase CysQ OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cysQ PE=2 SV=2	0.018	-0.075	-0.059	-0.054	0.112	0.149	
Dihydroxy-acid dehydratase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ilvD PE=3 SV=2	0.019	-0.153	-0.175	-0.154	-0.219	-0.178	
Putative hydrolase of the HAD superfamily OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ybhA PE=4 SV=1	0.019	-0.198	-0.299	-0.287	-0.414	-0.331	
Adenylosuccinate lyase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=purB PE=1 SV=1	0.02	0.284	0.197	0.299	0.117	0.192	
Multidrug resistance secretion protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=emrA PE=4 SV=1	0.02	-1.289	-1.347	-1.043	-0.881	-0.538	
Transcriptional repressor of nag (N-acetylglucosamine) operon (NagC/XylR family) OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720)	0.02	0.353	0.352	0.241	0.253	0.249	

GN=nagC PE=4 SV=1							
Selenide, water dikinase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=selD PE=3 SV=1	0.02	-0.501	-0.506	-0.637	-0.460	-0.627	
50S ribosomal protein L6 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rplF PE=3 SV=1	0.021	-0.111	-0.172	-0.133	-0.414	-0.416	
Phosphoenolpyruvate carboxylase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ppc PE=3 SV=1	0.021	0.146	0.170	0.118	0.067	0.014	
Thiosulfate transporter subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=cysP PE=4 SV=1	0.021	-0.039	-0.013	-0.072	0.200	0.294	
Ribonuclease PH OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rph PE=3 SV=1	0.021	-0.208	-0.183	-0.279	-0.137	-0.107	
NADH dehydrogenase subunit E OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=nuoE PE=4 SV=1	0.021	-0.179	-0.210	-0.204	-0.358	-0.287	
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sucB PE=3 SV=1	0.022	0.082	0.051	0.071	0.146	0.208	
Glutaredoxin OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ydhD PE=3 SV=1	0.022	-0.138	-0.167	-0.190	-0.278	-0.222	
Glucose-1-phosphate cytidylyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rfbF PE=4 SV=1	0.022	0.176	0.138	0.179	0.189	0.254	
Glucosyltransferase I OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rfaG PE=4 SV=1	0.022	0.155	0.084	0.248	-0.013	0.009	
Response regulator in two-component regulatory system with NarX (Or NarQ) OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=narL PE=4 SV=1	0.022	-0.092	-0.163	-0.096	-0.375	-0.414	
Aminotransferase AlaT OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfbQ PE=4 SV=1	0.022	-0.167	-0.108	-0.194	-0.242	-0.365	
Phospho-2-dehydro-3-deoxyheptonate aldolase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=aroG PE=3 SV=1	0.022	-0.255	-0.254	-0.268	-0.393	-0.415	
2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ubiF PE=4 SV=1	0.022	0.391	0.394	0.311	0.368	0.361	
Spermidine N1-acetyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=speG	0.022	0.261	0.235	0.284	0.372	0.604	

PE=4 SV=1							
tRNA-modifying protein YgfZ OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ygfZ PE=3 SV=1	0.023	0.202	0.180	0.193	0.160	0.158	
L-seryl-tRNA(Sec) selenium transferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=selA PE=3 SV=1	0.023	0.126	0.203	0.157	0.382	0.339	
Transcriptional regulator SlyA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=slyA PE=3 SV=1	0.023	-0.215	-0.243	-0.232	-0.254	-0.206	
23S rRNA (guanosine-2'-O-)-methyltransferase RlmB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=rlmB PE=3 SV=1	0.023	-0.147	-0.193	-0.328	-0.262	-0.285	
Homoserine kinase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=thrB PE=3 SV=1	0.023	-0.513	-0.506	-0.464	-0.175	-0.249	
Orotate phosphoribosyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pyrE PE=3 SV=1	0.024	-0.216	-0.239	-0.230	-0.087	-0.099	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ybfF PE=4 SV=1	0.024	-0.191	-0.166	-0.156	-0.057	-0.051	
Penicillin-binding protein 1B OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=mrcB PE=3 SV=1	0.024	-0.142	-0.041	0.014	0.162	0.127	
Threonine synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=thrC PE=4 SV=1	0.024	0.386	0.283	0.286	0.083	0.102	
Putative S-adenosylmethionine/tRNA-ribosyltransferase-isomerase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_1868 PE=4 SV=1	0.024	0.146	0.136	0.138	0.003	-0.054	
L-threonine aldolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ltaA PE=4 SV=1	0.024	-0.222	-0.182	-0.172	-0.089	-0.114	
Outer membrane lipoprotein Blc OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=blc PE=4 SV=1	0.024	0.384	0.368	0.370	0.261	0.371	
UPF0149 protein YgfB OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ygfB PE=3 SV=2	0.024	-0.364	-0.307	-0.299	-0.180	-0.126	
Iron-sulfur cluster assembly scaffold protein IscU OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nifU PE=3 SV=1	0.025	-0.575	-0.609	-0.589	-0.664	-0.676	
Uncharacterized protein YjaG OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yjaG PE=4 SV=1	0.025	0.401	0.336	0.316	0.435	0.438	

D-glycero-beta-D-manno-heptose-1,7-bisphosphate 7-phosphatase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=gmhB PE=3 SV=1	0.025	-1.047	-0.885	-0.928	-0.845	-0.855	
Transcriptional regulatory protein TyrR OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tyrR PE=3 SV=1	0.026	0.091	0.062	0.152	0.017	0.073	
Chaperone SurA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=surA PE=3 SV=1	0.026	-0.189	-0.208	-0.200	-0.114	-0.063	
3-dehydroquinate synthase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=aroB PE=3 SV=1	0.026	0.144	0.046	0.084	-0.053	-0.023	
Putative phosphoglucomutase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yqaB PE=4 SV=1	0.026	0.012	-0.076	0.108	0.467	0.336	
10 kDa chaperonin OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=groES PE=3 SV=1	0.027	0.043	-0.052	0.015	0.150	0.238	
Arginine--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=argS PE=3 SV=1	0.027	0.033	0.023	0.032	0.151	0.114	
D-3-phosphoglycerate dehydrogenase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=serA PE=3 SV=1	0.027	0.293	0.285	0.284	0.031	0.048	
Putative enzyme OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yihX PE=4 SV=1	0.027	-0.188	-0.163	-0.154	-0.193	-0.208	
Glyoxylate/hydroxypyruvate reductase B OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ghrB PE=3 SV=1	0.027	0.137	0.199	0.125	0.277	0.365	
Putative inner membrane protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yfgM PE=4 SV=1	0.027	-0.797	-0.822	-0.668	-0.561	-0.830	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yahO PE=4 SV=1	0.027	0.776	0.725	0.676	0.749	0.824	
Isovaleryl CoA dehydrogenase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=aidB PE=3 SV=1	0.027	0.733	0.795	0.646	0.820	0.868	
Fe/S biogenesis protein NfuA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nfuA PE=3 SV=1	0.028	-0.299	-0.206	-0.301	-0.221	-0.207	
UPF0482 protein YnfB OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ynfB PE=3 SV=1	0.028	-0.046	-0.062	-0.003	0.307	0.400	
Uridylate kinase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pyrH PE=1 SV=1	0.029	-0.072	0.025	-0.003	0.342	0.302	

Bifunctional protein GlmU OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=glmU PE=3 SV=1	0.029	-0.119	-0.154	-0.146	-0.139	-0.079	
Flagellar motor switch protein FliM OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fliM PE=3 SV=1	0.029	-0.045	0.067	-0.144	0.003	0.036	
Carbonic anhydrase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=PSLT046 PE=3 SV=1	0.029	-0.912	-0.370	-0.864	-0.473	-0.623	
Putative transcriptional regulator OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_5307 PE=4 SV=1	0.029	0.386	0.355	0.295	0.323	0.407	
Ribosomal RNA small subunit methyltransferase G OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=gidB PE=3 SV=1	0.029	0.168	0.136	0.367	0.204	0.186	
Protein-L-isoaspartate O-methyltransferase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pcm PE=3 SV=1	0.029	-0.155	-0.295	-0.194	-0.012	-0.024	
Exopolyphosphatase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=ppx PE=3 SV=2	0.03	-0.205	-0.160	-0.267	-0.322	-0.265	
Periplasmic disulfide isomerase, thiol-disulphide oxidase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=dsbG PE=4 SV=1	0.03	-0.396	-0.261	-0.334	-0.308	-0.350	
tRNA/tmRNA (uracil-C(5))-methyltransferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=trmA PE=2 SV=2	0.03	-0.474	-0.472	-0.405	-0.206	-0.311	
Esterase FrsA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=frsA PE=3 SV=1	0.031	0.129	0.146	0.161	0.137	0.134	
Periplasmic murein tripeptide transport protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=mppA PE=4 SV=1	0.031	-0.341	-0.293	-0.309	-0.241	-0.145	
Ornithine decarboxylase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=speF PE=4 SV=1	0.031	-0.347	-0.248	-0.225	-0.222	-0.394	
dTDP-4-dehydrorhamnose reductase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=rfbD PE=4 SV=1	0.031	-0.041	-0.134	-0.149	-0.189	-0.136	
Putative sugar transport protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yneA PE=4 SV=1	0.032	0.126	-0.116	0.035	0.208	0.071	
UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=murF PE=3 SV=1	0.032	0.525	0.584	0.519	0.508	0.460	
Putative periplasmic binding protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=sitA PE=3 SV=1	0.032	0.028	0.345	-0.064	0.406	0.453	

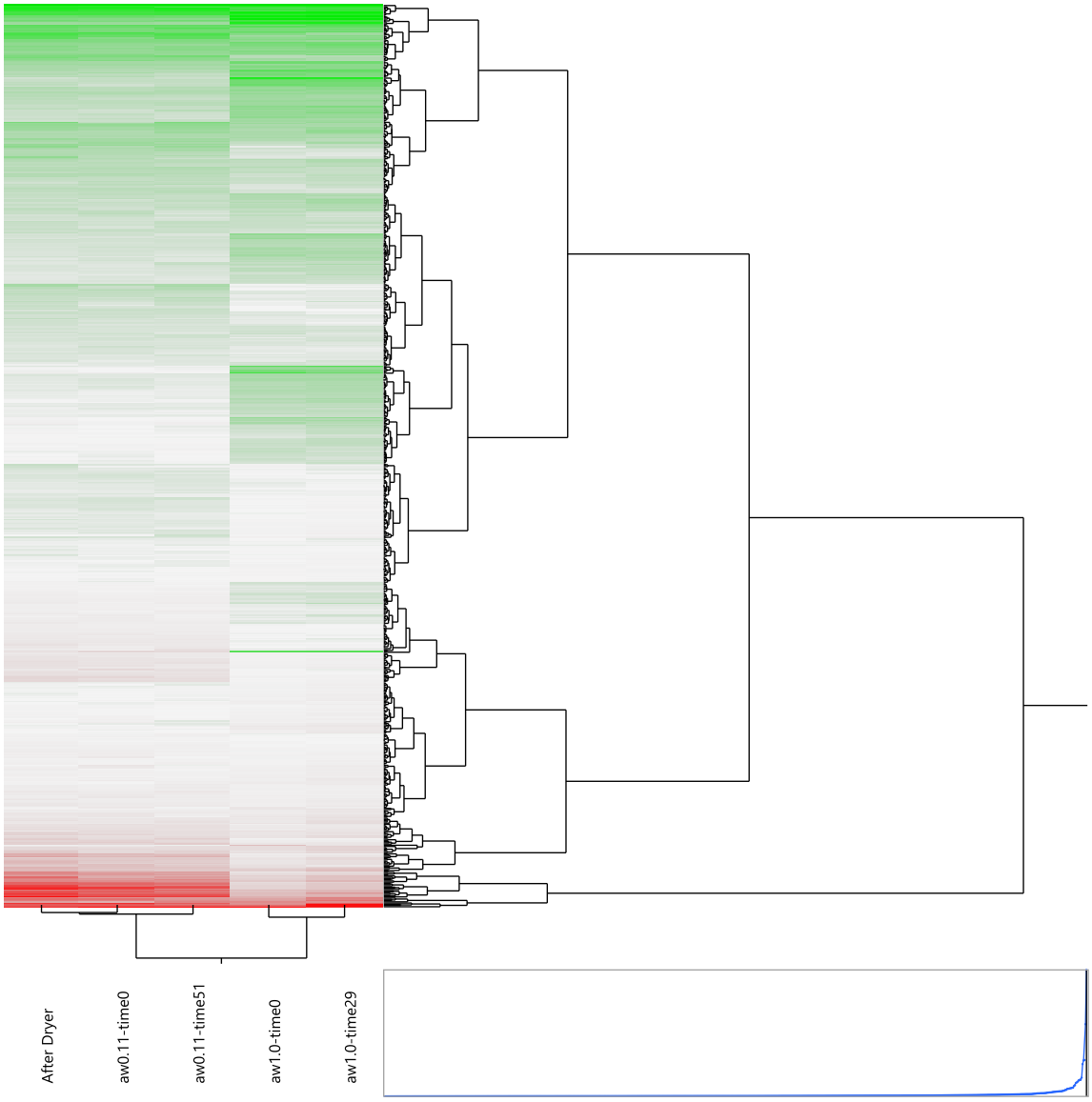
Tat proofreading chaperone DmsD OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ynfI PE=3 SV=1	0.032	0.099	0.129	-0.156	0.408	0.352	
GTPase Der OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=der PE=1 SV=2	0.033	0.078	0.115	0.051	-0.095	-0.125	
Putative glutathione S-transferase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yibF PE=4 SV=1	0.033	0.198	0.175	0.181	0.098	0.129	
Uncharacterized protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_3219 PE=4 SV=1	0.033	-0.134	-0.128	-0.125	-0.443	-0.455	
G/U mismatch-specific DNA glycosylase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=mug PE=3 SV=1	0.034	-0.273	-0.222	-0.149	-0.175	-0.091	
S-formylglutathione hydrolase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fghA PE=3 SV=1	0.034	-0.033	0.014	-0.130	0.315	0.396	
Putative outer membrane lipoprotein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yajI PE=4 SV=1	0.034	-0.185	-0.228	-0.251	0.004	0.054	
Dihydroorotate dehydrogenase (quinone) OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=pyrD PE=3 SV=2	0.035	-0.148	-0.179	-0.222	-0.117	-0.093	
Transcriptional repressor OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yiaJ PE=4 SV=1	0.035	0.274	0.078	0.450	0.161	0.304	
Putative translation factor OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yciO PE=4 SV=1	0.036	-0.123	-0.061	-0.094	0.149	0.066	
Cell division protein ZipA OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=zipA PE=3 SV=1	0.036	-0.003	-0.107	-0.137	0.533	0.536	
Bifunctional chorismate mutase/prephenate dehydratase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=pheA PE=4 SV=1	0.036	0.371	0.290	0.291	0.238	0.313	
Aldose 1-epimerase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=galM PE=3 SV=1	0.036	0.186	0.272	0.436	-0.063	-0.074	
UPF0434 protein YcaR OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ycaR PE=3 SV=1	0.036	-0.969	-0.878	-1.054	-0.862	-0.953	
UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=murE PE=3 SV=1	0.037	0.181	0.279	0.038	0.308	0.302	
Tyrosine--tRNA ligase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tyrS PE=3 SV=1	0.038	0.023	0.078	-0.032	-0.065	-0.175	
30S ribosomal protein S14 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262)	0.038	-0.333	-0.352	-0.382	-1.088	-1.011	

GN=rpsN PE=3 SV=1							
Alkyl hydroperoxide reductase F52a subunit OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ahpF PE=3 SV=1	0.04	-0.126	-0.171	-0.205	-0.169	-0.282	
Fused phosphoenolpyruvate-protein phosphotransferase PtsP/GAF domain-containing protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=ptsP PE=3 SV=1	0.04	0.495	0.425	0.451	0.289	0.309	
Glutaredoxin 2 OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=grxB PE=4 SV=1	0.042	-0.081	-0.102	-0.121	-0.172	-0.240	
Putative transcriptional regulator OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_5136 PE=4 SV=1	0.043	0.289	0.217	0.356	0.348	0.388	
Putative catalase OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=STM14_2094 PE=4 SV=1	0.043	1.279	0.952	1.108	1.178	1.321	
Bifunctional protein PutA OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=putA PE=2 SV=4	0.044	0.312	0.307	0.284	0.239	0.332	
Putative transport protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yhbS PE=4 SV=1	0.044	-0.227	-0.068	0.044	0.068	0.192	
Putative oxidoreductase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM0564 PE=3 SV=1	0.044	0.140	0.239	0.137	0.452	0.569	
HTH-type transcriptional repressor NsrR OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nsrR PE=3 SV=1	0.046	0.134	0.051	0.103	-0.116	-0.007	
Inducible lysine decarboxylase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=cadA PE=3 SV=1	0.047	-0.095	-0.095	-0.091	-0.021	-0.053	
Putative ABC-type transport system ATPase component OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yejF PE=3 SV=1	0.047	0.524	0.534	0.431	0.364	0.311	
UPF0502 protein YceH OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yceH PE=3 SV=1	0.047	-0.306	-0.305	-0.312	-0.128	-0.153	
Biofilm formation regulatory protein BssR OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=bssR PE=4 SV=1	0.048	-0.708	-0.596	-0.752	-0.597	-0.462	
Putative glycohydrolase OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=yegU PE=4 SV=1	0.048	0.128	0.141	0.130	0.273	0.221	
Endonuclease III OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=nth PE=3 SV=1	0.048	-0.302	-0.264	-0.442	-0.588	-0.648	
CinA-like protein OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=STM2293 PE=3 SV=1	0.049	0.255	0.180	0.141	0.271	0.274	

Tol protein, role in outer membrane integrity OS=*Salmonella* Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=tolR PE=3 SV=1	0.049	-0.034	-0.021	-0.099	0.080	0.098	
Ferric uptake regulator OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=fur PE=3 SV=1	0.05	-0.263	-0.148	-0.233	-0.431	-0.409	
Cell division protein OS=*Salmonella* Typhimurium (strain 14028s / SGSC 2262) GN=yhjQ PE=4 SV=1	0.05	0.057	0.120	0.271	0.410	0.532	

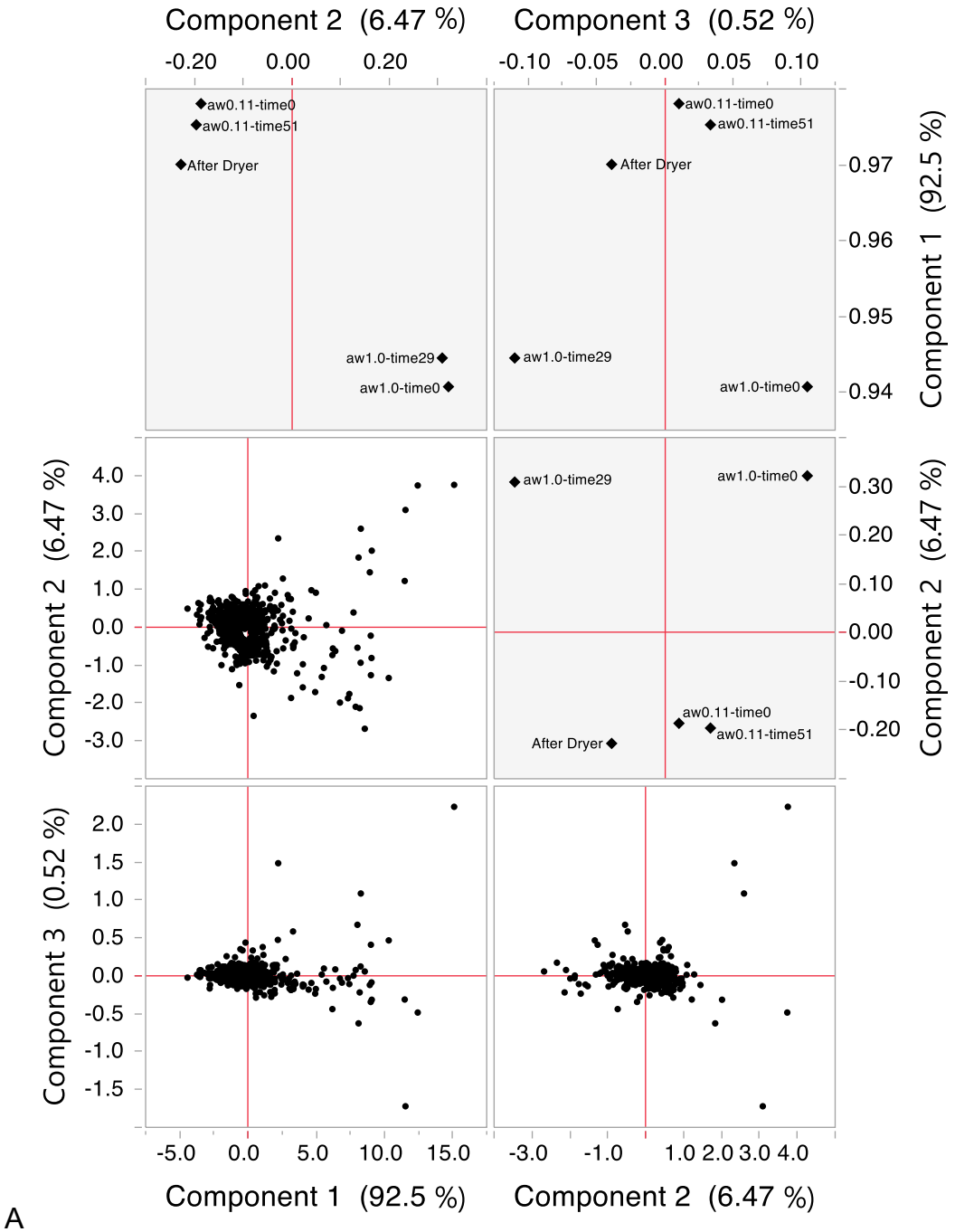
Appendix 5. Two-way hierarchical clustering of the 734 differentially expressed proteins.

The clustering was based on the principal component 1 indentified by the first PCA.

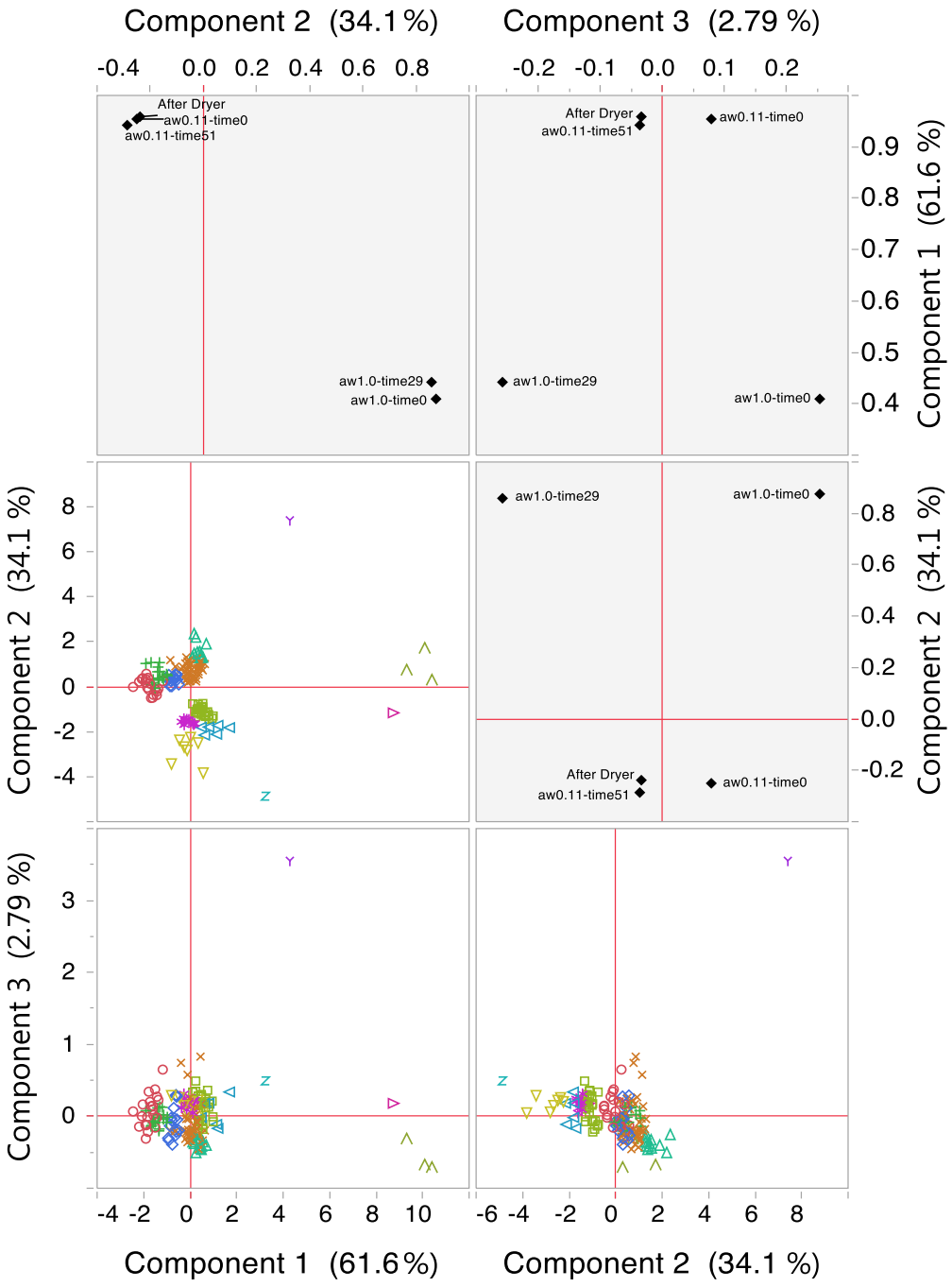


Appendix 6. PCA plots for the 734 differentially expressed proteins (A) and for the final group of 175 proteins (B).

The plots show the distribution of the samples of *Salmonella* and the proteins based on the combination of the first three components identified.



A


Component 2 (34.1 %)
Component 3 (2.79 %)
After Dryer
aw0.11-time0
aw0.11-time51
aw1.0-time29
aw1.0-time0
Component 1 (61.6 %)
Component 2 (34.1 %)
Component 3 (2.79 %)
Component 1 (61.6 %)
Component 2 (34.1 %)

B